CONTRACT REPORT

Part II

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PRINCIPAL INVESTIGATOR:	William F. Serat, Ph.D

COMMUNITY STUDIES ON PESTICIDES

Contract Report # 23 Covering the Period
November 15, 1969 to November 1, 1970

State of California
Department of Public Health
Bureau of Occupational Health

PART II

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WORK UNIT # VII - PESTICIDE POISONINGS AND ACCIDENTS

I - A) POISONINGS AMONG FARM LABORERS

Three confirmed outbreaks of organic phosphate poisoning occurred among crews of Tulare County farm laborers during 1970. In addition, a suspected but never confirmed case in Tulare County and a serious outbreak in Kern County were brought to the attention of the staff of Pesticide Project.

All of the cases involving Tulare County crews occurred among citrus pickers. In two of the three cases, we were notified by the Tulare County Agricultural Commissioner's Office, within hours after the laborers began arriving at local hospitals or physicians' offices. The rapid reporting is due at least in part to a local awareness of the need for better communication between public agencies and private physicians in the county. As described elsewhere in this report, members of the Pesticide Project field staff have for the last two years been conducting studies upon the health of Tulare County farm laborers. Their presence in the county has also contributed to the rapidity of notification as well as the completeness of the epidemiological information gathered. The information developed regarding the three outbreaks of poisoning among Tulare County farm laborers is summarized as follows:

1. Terra Bella - May 25th to May 28th, 1970

The first outbreak occurred between May 25th and May 28th and actually involved crews employed by three separate citrus packing houses and working in three separate areas near Terra Bella in the citrus region of Tulare County. Much of the following data was obtained by the staff of the Tulare County Agricultural Commissioner's Office, to whom we owe a considerable debt of gratitude.

a) In a crew of 20 working for the Waddell and Sons Packing House of Lindsay, California, two laborers became seriously ill while picking navel oranges the morning of May 25th. The crew was picking on property owned by James R. Sharer. One laborer, a Mr. F.S., reported to Dr. C. Mc. at 1:00 P.M. The doctor reported to the investigator that, upon first observation, the patient was nauseated, weak and sweaty. He was given emergency treatment for organic phosphate poisoning, including atropine sulfate and blood was drawn for a test of cholinesterase activity. The patient was told not to return to work for six to eight days. The results of the cholinesterase tests, which were done by a local Lindsay, California laboratory using the delta pH method, were as follows:

INITIAL	RED BLOOD CELLS	PLASMA
Mr. F.S.	.15	.05
Normal Range	0.55-1.25	0.41-1.65

Mr. A. De L. reported to a local clinic at 3:00 P.M. on May 25th. He was seen by Dr. D.B., and according to the investigator, told the doctor that he had vomited eight times, had body numbness, no saliva and was sweating profusely. The doctor gave the patient atropine sulfate and drew blood for a test of cholinesterase activity. Blood for the same purpose was drawn upon upon Mrs. De L., who had worked together with her husband in the same crew and had accompanied him to the clinic. She had however, according to the doctor shown no outward symptoms of poisoning. The cholinesterase determination was made by a Visalia, California Laboratory using the pH Stat method. The laboratory reported the following results:

	RED BLOOD CELLS	PLASMA
Mr. De L.	7.92	0.76
Mrs. De L.	8.85	0.81

The clinic considers anything less than 8.00 for cells and 2.00 for plasma to be abnormally low. The patient was advised not to return to work for 7 days.

In both cases, the doctors considered these to be definite cases of organic phosphate poisoning. The Sharer property had been sprayed with a mixture of parathion and ethion during the eighth and eleventh of May, approximately half of the acreage being treated each day. The pesticide treatment was as follows:

Parathion 7½ pounds per acre of 25% wettable powder

Ethion 15 pounds per acre of 25% wettable powder

The Waddell crew had begun working on the Sharer property on May 25th, the morning they became ill and reported to the physicians. On May 21st and 22nd, they had also picked oranges owned by Mr. Stan Peterson. These had last been sprayed on March 29, 1970 with Parathion. There is no record of the crew members or of Mr. S. or Mr. F. in particular, having worked on May 23rd or May 24th.

On May 27th, two days after the workers reported to the physicians, leaf and fruit samples were taken from the Sharer property for residue analysis by the California Department of Agriculture. That Department's report of the results of those tests is found on the following page. Each sample represents a composite of leaves or fruit from several trees.

	DATE TREATED	DATE SAMPLED	PARTS PER MILLION Parathion	Ethion	Others
Leaf Sample #1	May 8th	May 27th	Trace	7.2	None
Leaf Sample #2	May 11th	May 27th	Trace	9.4	None
Oranges	May 8th	May 27th	Trace	0.1	None

b) A crew working for the Southern Tulare County Citrus Association was also involved in an outbreak of organic phosphate poisoning during the latter part of May. Although the Association provided a list of 57 names and address of workers, it was determined that the particular crew in which the outbreak occured totaled 12 workers. Eleven of these became ill on May 28th while picking oranges on property owned by the B and W Ranch.

On the evening of May 28th, eight of the crew members were taken by their foreman to Dr. H. of Lindsay, California. He described them as showing symptoms that might indicate organic phosphate poisoning. He provided emergency treatment which included atropine sulfate and drew blood for cholinesterase determinations. According to the doctor, all the workers responded to the emergency treatment. These workers were all young Spanish speaking males in their twenties or early thirties.

The blood samples drawn by Dr. H. were mailed to an out-of-state laboratory for cholinesterase determinations. This laboratory using a method described as being by "de la Huerga *et al*" reported the following results. Only serum cholinesterase levels were reported by the laboratory.

PATIENT'S INITIAL	SERUM UNITS
E.P.	Not Reported
L.C.	26
R.A.	Not Reported
P.L.	39
R.A.	26
F.L.	26
J.S.	26
A.D.	39

According to instructions from the laboratory, normal serum values are 130 to 310 units.

An additional Spanish speaking crew member and his two female children who had accompanied him into the groves went to a local clinic and were seen by Dr. D.B. He reported to the investigator that they showed signs of organic phosphate poisoning and had responded well to treatment. Blood was drawn for cholinesterase determinations. The results of those tests, using the pH Stat method are as follows:

INITIAL	RED BLOOD CELLS	PLASMA
A.P. (Male)	6.51	0.56
O.P. (Female)	11.57	0.54
J.P. (Female)	15.25	0.71

These tests were made for the same clinic as in a) above. They consider any level below 8.00 for cells or 2.00 for plasma to be abnormally low.

It was determined by the investigator from the Tulare County Agricultural Commissioner's Office that the B and W property had been treated with a mixture of ethion, guthion and zinc sulfate in 1200 gallons of water per acre on May 14th, 15th, and 16th. The pesticide treatment was as follows:

Ethion	16 pounds per acre of 25% wettable powder
Guthion	6 pints per acre of 2 pounds per gallon emulsifiable concentrate
Zinc Sulfate	8 pounds per acre

The Southern Tulare County Citrus Association crew began picking on the B and W property on May 26th, the day before they became ill and reported to the physicians. On May 26th and early on the 27th, they had picked oranges on another B and W property, this one treated on May 13th, 14th and 15th with a mixture of kelthane, guthion, zinc sulfate and urea in 1000 gallons of water per acre. The treatment was as follows:

Kelthane	10 pints per acre of 4 pounds per gallon emulsifiable concentrate

Guthion	6 pints per acre of 2 pounds per gallon emulsifiable concentrate
Zinc Sulfate	8 pounds per acre
Urea	50 pounds per acre

Leaf and fruit samples were taken on May 29th from the groves in which the workers were picking when they became ill on the previous day. The report of the results of analysis for pesticide residues on those leaves indicates no residue on the fruit and quantities of ethion on the leaf samples ranging from a trace to 7.0 parts per million. No residues of guthion were detected.

c) A third crew of farm laborers was involved in the outbreak of organic phosphate poisoning in the Terra Bella area in late May, 1970. This crew, consisting of 19 males and females with Spanish surnames, was employed by the Lindsay Fruit Association.

One female worker reported to Dr. C. Mc. on May 28th. The doctor reported that the patient was nauseated and had symptoms of phosphate poisoning. A check of cholinesterase indicates the following results. The determination was made in a local Lindsay Laboratory using the delta pH method.

INITIAL	RED BLOOD CELLS	PLASMA
S.R. (Female)	0.60	0.40
Normal Range	0.55-1.25	0.41-1.65

The crew had begun picking fruit on the property owned by W.A. Thompson on May 25th, 1970. On May 16th, the area had been treated with a mixture of guthion, Delnav and Sorba spray in the following concentrations:

Guthion	1 gallon per acre of 2 pounds per gallon emulsifiable concentrate
Delnav	1 gallon per acre of 8 pounds per gallon emulsifiable concentrate
Sorba Spray	3 pints per acre

The above mixture was applied in 250 gallons of water per acre. On March 31st, 1970, this property had also been treated with parathion, phosphamidon, zinc sulfate and urea.

Prior to May 25th, the same crew had been picking oranges in a grove owned by Mr. Gary Davis. This property had been treated with guthion, ethion and sulphur on April 18th, 1970.

Leaves were taken on May 29th from the Thompson grove for analysis of pesticide residues by the California Department of Agriculture. The results of the analysis are summarized as follows:

	PARTS PER MILLION		
	Guthion	Delnav	Others
Leaf Samples	6	80	None

The staff of the Pesticide project conducted a follow-up study of as many members from the three crews as possible. An attempt was made to contact each crew member except those who had previously reported to a physician as described above. A worker (Mr. E.P.) who had gone to a doctor and whose cholinesterase tests were incomplete was included in the study. In this manner, cholinesterase tests as well as occupational medical histories were obtained from 24 crew members. Cholinesterase determinations were made at the Mineral King Laboratory in Visalia using the pH Stat method. This laboratory has made all the cholinesterase determinations upon Tulare County farm workers described in Units I and III of this report. The results of those determinations are shown in Table I. They indicate that several days after the known exposure of the crews to organic phosphate insecticides many workers had abnormally low cholinesterase levels.

B) McFARLAND - SEPTEMBER 17, 1970

On September 18, 1970, the staff of the Pesticide Project was notified an outbreak of pesticide poisoning the previous day among a crew of 35 orange pickers in Northern Kern County near the Tulare County border. The first information about the incident reported the illness of 32 laborers and described the cause as due to parathion exposure. (See newspaper article, Figure 1). Additional information, as described below indicates that another material, delnav , was probably involved.

A member of the Pesticide Project staff conducted an immediate investigation in the area with the aid of staff members from the State Department of Agriculture and the Tulare County Agricultural Commissioner's Office. The latter was involved because the entire work crew of 35 came from the area of Lindsay, California in Tulare County. The Kern County Agricultural Commissioner provided assistance and made information regarding spray histories available to the investigators.

Table I

CHOLINESTERASE LEVELS IN MEMBERS OF THREE
TULARE COUNTY ORANGE PICKING CREWS

PACKING HOUSE EMPLOYER	PARTICIPANT'S INITIAL	AGE	SEX	DATE BLOOD WAS DRAWN	CHOLINESTERASE ACTIVITY IN uM/Min./ml	
					Plasma	RBC
LFA*	M.R.	Unk	Male	June 2	2.01	9.03
LFA	A.M.	Unk	Male	June 2	2.75	10.18
LFA	R.M.	66	Male	June 2	3.16	11.97
LFA	G.D.	29	Male	June 3	1.42	8.98
LFA	M.D.	36	Female	June 3	2.48	13.29
LFA	I.D.	14	Male	June 3	1.19	8.50
LFA	L.M.	69	Male	June 3	2.32	12.09
LFA	M.D.	74	Male	June 3	1.68	9.70
LFA	J.S.	18	Male	June 4	3.34	10.74
LFA	E.F.	43	Male	June 13	0.99	5.84
Wadd*	P.S.	47	Female	June 5	2.17	11.45
Wadd	P.D.	53	Female	June 8	1.35	6.75
Wadd	J.R.**	56	Female	June 9	0.73	6.75
Wadd	J.R.**	35	Male	June 9	0.78	6.23
Wadd	M.P.	31	Male	June 10	2.06	9.39
Wadd	J.Z.	43	Male	June 10	0.94	6.37
	Repeat			June 16	2.31	6.70
STCA*	L.D.	22	Male	June 13	3.23	11.76
STCA	I.D.	23	Male	June 13	2.59	9.41
STCA	R.D.	30	Male	June 13	2.75	9.29
STCA	V.M.	23	Male	June 13	1.76	9.52
STCA	C.M.	22	Female	June 13	3.11	12.35
STCA	G.S.	24	Male	June 13	3.23	11.61
STCA	B.S.	26	Female	June 13	3.71	11.76

* Packing Houses

LFA - Lindsay Fruit Association
Wadd - Waddell and Sons
STCA - Southern Tulare County Citrus Association

** Long Term Study Participants

At approximately 11:30 A.M. on September 17th, the first of 12 Spanish speaking male farm laborers were taken by their crew foreman to Dr. A.C. in McFarland. As additional cases appeared, the doctor had the patients transferred to the nearby Delano District Hospital. By 2:00 P.M., 12 workers had been admitted to the hospital. The doctor described the symptoms as cramps, vomiting, dizziness and pinpointed pupils. All patients were fed intravenously and all except one was treated with atropine. The most severe cases were also treated with Protopam. Blood samples were drawn from each patient for cholinesterase tests. Unfortunately, the blood was drawn some three hours after several of the patients had received Protopam. The blood was sent to a laboratory located in Bakersfield, some 40 miles away, for the cholinesterase determinations. The laboratory, using the delta pH method, reported the results shown in Table II.

The doctor described these as among some of the most serious cases of phosphate insecticide poisoning he had seen. Two of the workers were severely disoriented and nearly unconscious when brought to the hospital. The fact that those most severely ill had the highest cholinesterase values is to be expected considering these were the persons who received Protopam three hours prior to the drawing of the blood and about 18 hours prior to the determination of enzyme activity. The rapid *in vivo* and *in vitro* reactivation of enzyme activity is described by Durham and Hayes 1962[a]. According to that article, 2-PAM at an *in vitro* concentration of 10^{-5}M reactivates as much as 80 percent of the phosphate inhibited enzyme within one minute. Protopam works the same way -- perhaps more rapidly than 2-PAM, and the concentration of 10^{-5}M represents the approximate level of the material in the blood following therapeutic administration.

Beginning at 3:00 P.M. on September 17th, 22 additional pickers from the same crew reported to Dr. M.M. in Lindsay. Although this town is about 50 miles from where they had been working that morning, it is the location of their homes. Of the 22 workers, the doctor described the first eight who reported between 3:00 P.M. and 9:15 P.M. of the first day, as having the most severe symptoms. They were dizzy and vomiting but did not, according to the doctor display pinpointing of the pupils. The two persons showing the most severe symptoms were administered atropine and hospitalized. On the next day, 14 more crew members came to Dr. M.M. One of these brought her two year old daughter who had accompanied the crew into the grove. The doctor described those persons as having few, if any, signs of poisoning. None of the patients on either day were administered Protopam.

Blood samples were obtained from each worker and the child. They were analyzed for cholinesterase activity using the delta pH method at a Lindsay laboratory. The results of those tests together with certain information

[a] Durham, W.F. and Hayes, W.J. Jr. 1962. Organic Phosphorus Poisoning and its Therapy. Archives of Environmental Health 5, 21-47.

Table II

CHOLINESTERASE LEVELS IN FARM WORKERS
HOSPITALIZED FOR ORGANOPHOSPHATE
POISONING IN DELANO, CALIFORNIA
SEPTEMBER 17, 1970

WORKER'S INITIAL	CHOLINESTERASE ACTIVITY	
	Red Blood Cell	Plasma
M.D.*	0.74	0.74
S.M.*	0.52	0.50
D.M.	0.37	0.45
G.H.*	0.64	0.53
J.Z.	0.45	0.77
J.A.*	0.52	0.47
Y.M.	0.47	0.75
R.L.*	0.62	0.95
G.S.	0.34	0.45
S.A.	0.44	0.65
J.C.*	0.54	0.88
M.R.*	0.52	0.55
Normal Range	0.55 to 1.25	0.41 to 1.65

* These patients receive the cholinesterase reactivating agent, Protopam three hours prior to the drawing of blood for cholinesterase determinations.

about the patients are shown in Table III. The table also indicates the date and time the worker first appeared at the hospital and if he or she were hospitalized or sent home.

These data indicate that those persons seen by the doctor during the same day they first became ill in the field, generally, had the lowest red blood cell cholinesterase activity. Since these were the persons the doctor described as having symptoms of poisoning while those reporting the next day had few, if any, such symptoms, one may perhaps judge that a delta pH level of 0.35 for red blood cell cholinesterase activity is the point at which symptoms of organic phosphate poisoning began to appear. The levels of plasma cholinesterase activity do not correspond well with the symptomology. In fact, among those seen the first day, the two workers with lowest red blood cell activity and who were hospitalized had the highest plasma cholinesterase activity.

On September 17th, this crew of 35 orange pickers had entered Roberts and La Borde Ranch at 6:00 A.M. By 10:00 A.M., several workers were feeling ill. The property had been treated with parathion on August 11th, 12th, 13th and 14th at the following rate.

Parathion	36 pounds of 25% wettable powder in 3600 gallons of water per acre.

The same property was previously sprayed with a mixture of Delnav and sugar in 250 gallons of water per acre on May 13th. The rate of application that time was as follows:

Delnav	4 pints of 8 pounds per gallon emulsifiable concentrate
Sugar	10 pounds

The same work crew had also picked oranges on another property, the Butte Vangel Ranch, during the period of September 10th to the 16th. This grove was treated with Delnav at the same rate and on the same date as listed above. No parathion was applied.

Field investigators of the California Department of Agriculture obtained leaf and fruit samples from both the Roberts - LaBorde and the Butte Vangel Ranches on September 18th, one day after the workers became ill and were first reported to the physicians. The result of the laboratory analysis of these samples is as follows:

			PARTS PER MILLION	
RANCH	DATE WORKERS PICKED	DATE SAMPLED	Delnav	Parathion
Butte Vangel	September 10th, 14th, 15th, 16th	18th	14.3 (leaves) (Range of Samples 3.4-21.3)	0 (leaves)

Fig. 1

The Bakersfield Californian
Sept. 18, 1970

Parathion Gets Blame for Illness

32 Recovering After Incident

Thirty-two field workers were recovering either at home or in the hospital today following apparent parathion poisoning Thursday while picking oranges on the Roberts and LaBorde Ranch just south of McFarland.

Twelve of the workers, all Mexican-American members of Cesar Chavez' United Farm Workers Union, were listed in satisfactory condition this morning at Delano Hospital. It was expected they would be released later today.

Two more workers were released this morning from Lindsay District Hospital where an additional six others were treated and released Thursday.

According to sheriff's deputies, the men were picking oranges for Paramount Citrus Growers. They started picking at 6 a.m. and by 10 a.m. they became dizzy and were vomiting.

Deputies said the property was sprayed for "red scale" Aug. 11 and posted "stay out." Tolerance time, according to deputies, is 30 days.

All spraying for red scale is handled by the state Red Scale District. The spraying on the Roberts and LaBorde ranch had been contracted by the state to Fisher, Randolph and Fisher of Delano.

The 12 workers were transferred to Delano Hospital after first being taken to a McFarland doctor by their foreman, Noe Del Bosque. The doctor then sent them to the hospital for treatment.

Of the other reported twenty victims, eight went to Lindsay District Hospital where two were admitted overnight.

Larry Itliong, assistant to Chavez said the union did not have enough information to issue a formal statment on the

see Parathion—page 14

from page 13

incident. All the striking workers are from Lindsay.

Following are the 12 workers admitted to Delano Hospital:

Jose Alvarado, 21, 946 Fresno Street, Delfino Morellon, 30, 986 Fresno Street; Jesus Zauala, 30, 986 Fresno Street; Ysidro Magana, 22, 991 Fresno Street; Samuel Aguilera, 25, 991 Fresno Street; Reynaldo Lopez, age unknown, 991 Fresno Street; Jose Cueves, 20, address unknown; Guillerno Ionia, 16, 946 Fresno Stret; Guillermo Hernandez, 33, 946 Fresno Street; Manuel Delayo, 38, 452 Tulare Road; Simon Magana, age unknown, address unknown; Manuel Reynoso, 42, 841 Fresno Street.

Those admitted and released today at Lindsay Hospital: Luis Mata, 19, 838 West Tulare Road and Ruben Macias Duran, 17, 1077 Denver.

Those treated and released at Lindsay Hospital were: Salvadore Mata, 20, 838 West Tulare; Hortencia Reynoso, 19, 841 Fresno Street; Pablo Sandoval Yniguez, 39, 977 Fresno Street Jose Gutierrez Duran, 38, 1077 Denver; Ubaldo Yavala, 34, 991 Fresno Street; and Ernesto Sorta, 41, 946 Fresno Street.

56 # lac - lacre
Aug 11 completed Aug 14, 1970

Table III

CHOLINESTERASE LEVELS IN FARM WORKERS TREATED
FOR ORGANOPHOSPHATE POISONING IN
LINDSAY, CALIFORNIA
SEPTEMBER 17th AND 18th, 1970

DATE	TIME FIRST SEEN (ALL PM)	INITIAL	AGE	SEX	DISPOSITION	CHOLINESTERASE ACTIVITY	
						Red Blood Cell	Plasma
September 17							
	3:00	S.M.	20	Male	Home	0.20	0.60
	3:20	L.M.	19	Male	Hospital	0.25	0.45
	3:20	H.R.	19	Female	Home	0.20	0.70
	3:40	R.D.	17	Male	Hospital	0.30	0.50
	3:45	P.Y.	39	Male	Home	0.35	0.40
	3:45	J.D.	38	Male	Home	0.55	0.45
	5:45	U.Y.	34	Male	Home	0.30	0.30
	9:15	E.S.	41	Male	Home	0.35	0.40
September 18							
	2:30	C.S.	2	Female	Home	0.60	0.80
	2:30	S.S.	23	Female	Home	0.50	0.45
	2:35	B.C.	53	Male	Home	0.45	0.50
	2:30	R.S.	16	Female	Home	0.45	0.45
	2:35	Y.D.	44	Male	Home	0.40	0.40
	2:30	J.S.	26	Male	Home	0.45	0.55
	2:30	F.C.	54	Male	Home	0.40	0.35
	2:45	A.M.	50	Male	Home	0.70	0.80
	2:30	M.S.	21	Female	Home	Not tested	
	2:30	I.S.	17	Female	Home	0.55	0.60
	7:00	J.M.	23	Male	Home	0.45	0.45
	7:00	M.E.	38	Male	Home	0.45	0.45
	7:00	D.M.	38	Male	Home	Not tested	
	9:20	A.C.	48	Male	Home	Not tested	
	9:30	J.Z.	29	Male	Home	0.50	0.50

Roberts & LaBorde	September 17th	18th	0.15 (Fruit) 10.4 (leaves) (Range of Samples 7.3-14.8)	0.15 (Fruit) 1.4 (Leaves) (Range of Samples 1.0-2.1)

C) ORANGE COVE, OCTOBER 1 AND 8, 1970

On October 1st and 8th, members of two different orange picking crews employed by the Orange Cove Citrus Association became ill while working on the Herman Bear Ranch in Northern Tulare County.

Eight members of a 20 worker crew became ill on October 1st and reported to physicians in the towns of Reedley and Orange Cove. In Reedley, Dr.L. reported that he saw four persons from that crew beginning about 1:00 P.M. on October 1st. They had nausea, were vomiting and had diarrhea. They responded to treatment and were sent home. The Doctor's First Report of Work Injury, filed as a requirement of the California Department of Industrial Relations, lists the causes of injury in three cases as "Probable exposure to pesticides". The other worker was described as having "Gastroenteritis". Cholinesterase tests were not made upon those reporting to the doctor on the first day. The next day, however, when three additional crew members described as having milder symptoms reported to the doctor, he did draw blood samples for cholinesterase determinations. The determinations were made in a Fresno laboratory using the delta pH method. The results of those determinations are as follows:

INITIAL	RED BLOOD CELLS	PLASMA
G.F.	0.68	0.40
J.G.	0.70	1.50
H.A.	1.25	1.48
Normal Range	0.55-1.25	0.41-1.65

Also on October 2nd, one of the crew members reported to Dr. R.S. in the town of Orange Cove. He told the doctor that he had been nauseated and vomited 10 to 12 times since picking oranges the previous morning. The doctor described the patient's symptoms as indicating organic phosphate insecticide poisoning. The man was hospitalized and blood drawn for cholinesterase determinations. Results of those tests are as follows:

INITIAL	RED BLOOD CELLS	PLASMA
E.E.	.15	0.2
Normal Range	0.55-1.25	0.41-1.65

One week later, on September 9th, the worker, who by then was released from the hospital, returned for another cholinesterase test. The results are as follows:

INITIAL	RED BLOOD CELL	PLASMA
E.E.	0.30	0.55
Normal Range	0.55-1.25	0.41-1.65

On October 8th, members of another crew became ill while picking oranges in another section of the same grove. Two men in a crew of 34 reported to Dr. R.S. in Orange Cove with symptoms of organic phosphate poisoning. The doctor indicated that one (Mr. U.E.) was nauseated, that his chest felt tight and that he had excessive salivation and perspiration. He responded well to treatment which included atropine and was told not to return to work for one week. Cholinesterase levels were as follows:

INITIAL	RED BLOOD CELL	PLASMA
U.E.	0.33	0.40
Normal Range	0.55-1.25	0.41-1.65

The other worker reporting to Dr. R.S. was the crew's fork lift operator. His symptoms were less severe and, according to the doctor, his cholinesterase levels indicated he was only slightly if at all, affected by the pesticide.

The Herman Bear Ranch had been treated with a mixture of parathion and malathion in 1200 gallons of water per acre on August 29th and 30th. The rate of application was as follows:

Parathion	12 pounds per acre of 12.5% wettable powder
Malathion	12 pounds per acre of 18.75% wettable powder

On October 9th, the California Department of Agriculture representatives secured foliage and fruit samples from the Bear property. These materials were analyzed for chlorinated hydrocarbon and organic phosphate pesticide residues. One major peak was found in each sample using both electron capture and phosphorus sensitive detectors. At present, however, the peak remains unidentified.

D) PORTERVILLE, MAY 5, 1970

In this case, three farm laborers in a crew of 32 became ill while prunning small lemon trees near Porterville. The three obtained medical attention from a local physician. According to the doctor, one of the workers (Mr. J.R.) reported that he felt good until about an hour after getting home from work at 3:00 P.M. and then started feeling very weak with numb arms and legs. He said he had diarrhea and nausea and his mouth kept salivating heavily. He also reported to the doctor that he felt like fainting and could not breathe. The doctor's diagnosis was acute pesticide poisoning. Two other workers showed less severe symptoms and, in order to rule out pesticide poisoning, cholinesterase tests were performed on these men. The results of the tests upon these, and the more severe case (Mr. J.R.) are as follows:

INITIAL	RED BLOOD CELL	PLASMA
J.R.	10.08	3.08
A.T.	11.25	1.97
J.T.	10.00	2.36

Since the laboratory which made these cholinesterase determinations considers levels above 2.00 for plasma and 8.00 for red blood cells to be normal, it is difficult to confirm this as a case of organic phosphate poisoning.

The California Department of Agriculture determined that the grove of lemon trees had been sprayed only one day prior to the occurrence of the illness among the pruners. The materials applied were as follows:

Delnav	3 quarts per acre of 8 pounds per gallon emulsifiable concentrate
Dibrom	1 pint per acre of 8 pounds per gallon emulsifiable concentrate

The above mixture was applied to the trees in 250 gallons of water per acre on May 4th. On May 6th, the day after the workers reported to the physician, trees were sampled to determine residues on the leaves. The results are as follows:

	Dibrom	Delnav
Leaves	None	139 ppm

E) KERN COUNTY, OCTOBER 1, 1970

As this report was in preparation, the staff of the Pesticide Project learned of an outbreak of pesticide poisoning on October 1, 1970 among a crew of potato planters in Kern County. Twelve workers became ill and reported to a Bakersfield physician after they contacted Di Syston mixed with fertilizer as it was applied to the potato seeds in the planting process. Fragmentary information indicates several of the workers were hospitalized.

The staff of the Pesticide Project and the California Department of Agriculture are currently investigating this outbreak and a report of the findings will be included in the next Quarterly Report.

II - LEAD POISONING OF HORSES

During the first part of this year, one of the Project Staff participated with members of the Department of Public Health's Bureau of Occupational Health and Environmental Epidemiology in an investigation into the cause of death of horses over a 2 year period in a small area near Benicia, California. An epidemiological investigation determined 37 horses had died during this period and that lead poisoning was the cause of death. Environmental sources of lead in the area were determined to be an ore smelter, auto exhaust from a nearby freeway, an abandoned tetraethyl lead salvage dump and a railroad car burning and salvage operation.

Samples of air, soil and vegetation taken in the area have indicated that the ore smelter is the major source of the lead. Following considerable investigation by state and local air pollution agencies the national management of the smelter announced it would be closed at the end of this year.

III - PENTACHLOROPHENOL POISONING IN THE HOME

A member of the Project Staff investigated a case of human pentachlorophenol poisoning in the Sacramento area. A redwood home had been treated extensively inside and out with two coats of a wood sealer, containing 5 percent technical pentachlorophenol. The architect who designed the home stipulated the use in order, as he said, "to keep the wood from turning white". According to information obtained from the architect two other homes of his design had been similarly treated. Information about these additional homes were turned over to the Sacramento County Health Department.

The material applied was advertised as "Wood-Preservative" and as such, state law required that its use be registered with the California Department of Agriculture. Bringing this case to the attention of that department, we learned that such registration was never obtained and that the label on the can of sealer lacked appropriate warning statements regarding application within structures -- as would be required to obtain proper registration. That department is presently investigating the entire registration matter including a possible violation of the Agricultural Code.

Table IV

PENTACHLOROPHENOL LEVELS IN BLOOD, URINE
AND AIR SAMPLES TAKEN FROM A SACRAMENTO HOME AND ITS OCCUPANTS

PERSON	SAMPLE	DATE SAMPLES	PENTACHLOROPHENOL
Housewife	Blood	March 27	6.2 ppm
Housewife	Urine	March 27	0.5 "
Husband	Blood	March 27	1.8 "
Housewife	Blood	April 26	2.18 "
Housewife	Urine	April 26	0.16 "
Housewife	Blood	July 9	1.62 "
Housewife	Urine	July 9	0.10 "
Housewife	Blood	Nov. 6	0.40 "

HOUSE LOCATION	TOTAL AIR SAMPLE	DATE SAMPLES	PENTCHLOROPHENOL
Living Room	61.2 liters	April 2	.028 mg/m^3
Family Room	50.4 "	April 2	.031 "
Master Bedroom	54.3 "	April 2	.012 "
Living Room	29.4 "	Sept. 14	.0087 "
Family Room	44.0 "	Sept. 14	.0062 "
Master Bedroom	71.3 "	Sept. 14	.0087

The house had been treated as described above during construction late in November 1969. The couple moved in, early in January and reported that they immediately saw their plants wilt and die. They also noticed a strong unpleasant odor. The paint salesman advised that they keep their windows open to air out the house (in January ?). By early February the wife had lost 20 pounds from an initial 116 pounds. At that time the woman had seen a doctor who diagnosed her illness as asthma and bronchitis. By the time the family contacted our Department in late March, the wife reported her condition was was growing continually worse and that she had been forced to leave the home a few days earlier.

With the cooperation of a Sacramento medical Facility, Project personnel obtained blood and urine samples from the woman one week after she had left the home. A blood sample was also taken at that time from the husband who continued to spend a limited time in the house. It should be pointed out that the woman had become more ill since she spent more time at home whereas the husband as president of a national manufacturing concern, was often out of town for periods of a week at a time.

Air samples were taken in the home six days after the blood and urine sampling described above. Metered miniature fritted bubblers containing 10 ml of isooctane were used. Experience has shown this system to be between 70 and 90 percent efficient.

One month after leaving the home the woman returned. By this time she had regained her weight and lost the respiratory symptoms. One week after returning home another blood and urine sample were obtained for analysis. Results of the chemical analysis of blood, urine and air samples for pentachlorophenol content are indicated in Table IV.

In June and early July the owner had two coats of clear resin sealer applied to all wooden surfaces in and outside the home. Following this additional blood samples were taken from the woman on July 9th and November 6th. Additional air samples were taken in the home on September 14th. The results of these determinations are summarized in Table IV.

The data indicate a decrease in pentachlorophenol levels in the air within the home as well as in the woman's blood. There was a brief illness of the woman in early September which the doctor described as a reoccurrence of the symptoms of pentachlorophenol poisoning. The woman left the home for a 20 day period during which the symptoms disappeared and have not reappeared since she returned again to the home.

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WORK UNIT # VIII - QUALITY CONTROL OF BIOCHEMICAL DETERMINATIONS

All nonenzymatic quality control check samples, both assayed and unassayed, have been sent to participating laboratories. The writing of this report predates the receipt and analysis of results for the more recent check samples.

At the Atlanta meeting in March it was decided to supplement the quality control program with additional mailings of assayed specimens to the 15 Project laboratories. These additional samples appear to have been beneficial inasmuch as the interlaboratory range in values has been narrowed. Coefficients of variation of some tests show marked improvement, while others remain wide with no apparent explanation. Table I lists these coefficients calculated at this time.

During the year, 3,510 single test determinations were prepared and mailed to Project laboratories. Our laboratories have analyzed 480 assayed samples (48 samples, 10 x each) and 558 unassayed samples.

Dr. Margen will attend the Biochemistry Committee meeting in Atlanta during the week of January 25, 1971. Hopefully, some reevaluation of biochemical testing and methodology will derive from the Committee's efforts.

Table I

REVIEW OF COEFFICIENT OF VARIATION FOR
NON-ENZYMATIC CHECK SAMPLES 1970

TESTS	SERIES AND SPECIMEN NUMBERS											
	Ser. # 1 - 70		2 - 70		3 - 70		A1 - 70		4 - 70		A2 - 70	
	Spec. # 1	2	1	2	1	2	N	AB	1	2	N	AB
Urea Nitrogen	7%	5%	7%	11%	10%	3%	-	4%	8%	5%	4%	-
Cholesterol	7%	12%	8%	6%	6%	12%	-	9%	6%	8%	3%	-
Creatinine	13%	10%	10%	18%	9%	36%	-	10%	15%	8%	10%	-
Glucose	6%	7%	6%	6%	8%	7%	-	3%	9%	4%	5%	-
Phosphorus	7%	11%	7%	6%	14%	13%	-	7%	7%	7%	5%	-
Total Protein	5%	4%	4%	8%	5%	8%	-	1%	3%	2%	5%	-
Albumin	7%	7%	11%	11%	5%	9%	-	10%	8%	6%	6%	-
Uric Acid	8%	6%	7%	7%	5%	6%	-	5%	11%	7%	4%	-
Hemoglobin	2%	2%	11%	2%	4%	3%	-	2%	4%	4%	3%	-
Hematocrit	2%	3%	10%	2%	2%	2%	-	4%	5%	6%	2%	-
White Blood Cells	11%	8%	15%	10%	15%	12%	-	-	11%	10%	-	-
Urine Creatinine	7%	8%	11%	8%	11%	6%	-	7%	8%	8%	5%	-
Urine Phosphorus	10%	8%	11%	14%	8%	9%	-	5%	15%	11%	6%	-
P.S.P.	15%	12%	14%	16%	-	-	-	-	-	-	-	-

WORK UNIT # IX - MULTIPROJECT DATA PROCESSING

Under the direction of the Data Management Section in Atlanta, Georgia, this work unit consisted of the preparation of the long term data subset. Two steps were involved: (1) the transfer of the data from the long term study forms to the card layout as specified, (2) the keypunching of these data from the card layout to IBM data cards. The IBM data cards were then sent to the computer center at the University of Washington where analysis of this project's data as well as other projects' data subsets has been in progress.

A program to edit all the project's data was used by the Computer Center at the University of Washington. We received the computer printout of the program which was applicable to our data. Any unusual values or errors were checked and corrected if necessary before being returned to the computer center for further analysis. Presumably, from the final analysis and evaluation a new course of direction will be established for the test regimen given to long term participants.

OTHER ACTIVITIES

STUDIES IN ADDITION TO THOSE OF THE WORK UNITS

This writeup is presented for a number of reasons. First, we will describe the need, finally realized, for extensive field testing of pesticides prior to sale, under a definitive protocol based on scientific judgement. Though such protocol was presented to the commercial concerns conducting the field tests in many instances compromising the methodology led to minimal results. Second, we will show that established reentry times, however set in past times, are not adequate (or are being violated). Third, with more awareness and somewhat better reporting methods, we are not learning of extensive poisonings among farm work crews. In past years, probably due to the fear of lost jobs or other reprisals, such poisonings undoubtedly went intentionally unnoticed. In addition, upon request we are acting in service to the State of California in establishing experimental protocol, advising on methods, conducting an analysis of results and in consulting for the establishment of more realistic reentry times into treated fields. Studies such as these open the door to many possible work units and focus attention on the Community Studies as a most important service and consultant to the State of California.

Following an episode of poisoning in May among citrus workers (see writeup of Work Unit VII), guthion (Chemagro Co.) and ethion (FMC-Niagara Division), among other pesticides, were placed on a temporary 30 day reentry interval and the companies were asked to present data if they believed this waiting time inappropriate.

The following protocol and results are those of Niagara and are presented as given to us. Our analyses and comments follows:

CITRUS PICKERS WORKING IN TREES TREATED WITH ETHION FORMULATIONS

(Report of Niagara Chem. Div. FMC. Corp.)

As a result of reported illness of agricultural workers exposed to tree crops sprayed with a combination of Guthion and ethion, the California Director of Agriculture, on June 19, 1970, issued an emergency order placing ethion on the list of chemicals for which an application permit is necessary and requiring a 30-day interval after application before reentry into a citrus planting for picking or other activity that requires substantial contact with foliage. A hearing was to be held 120 days to determine whether the order would be continued in effect. A study was designed by Niagara Chemical Division to accumulate hard field, as well as laboratory data, to present at the hearing.

A suitable grove of valencia oranges was located near Lindsey, Tulare County. Ethion at the rate of 7 pounds active per acre was applied as EC and as WP to two 5 acre blocks of trees on July 24. Picking commenced on July 27 and continued through August 1.

The pickers, employees of the Euclid Packing Co., were interviewed for medical history, work history and current medical problems on July 24. Preliminary cholinesterase determinations were made on July 24 and July 25. Later determinations were made after one day's picking and after five day's picking. Blood samples could not be obtained on intervening days due to picker refusal. The cholinesterase determinations were made by Tulare Clinical Laboratories using a modification of the potentiometric method described by Nabb and Whitfield. Normal plasma values for this technique are 2.00 to 5.50 uM/ml/min for males and 1.20 to 4.00 for females. Normal erythrocyte values are 8.00 to 17.00 for males and 7.50 to 15.50 for females.

New cotton gloves were issued to five pickers in the EC block and five in the WP block at the start of the day on July 29. They were asked to wear them without washing them for three days. The gloves were picked up at the end of the work period on July 31. The left glove of each pair was placed in a half-gallon jar and aggitated in 500 ml of benzene for 30 minutes. The extraction was repeated twice, using 100 ml portions of benzene. The extracts were filtered through anhydrous sodium sulphate. Analysis was by use of a Tracor Model MT-220Q gas chromatograph using a 2 foot x ¼ inch Pyrex column packed with 10% DC-200 coated on 80/100 inch acid washed chromosorb W.H.P. A Melpar Flame Photometric Detector using a 526 micron filter was used to quantitate the residues.

Air samples were taken by use of personnel air samplers with the collection filter in the breathing zone of the picker. One picker in the EC block and one in the WP block were monitored each day on July 29 and July 30. The samples were collected on Gelman GA4 metricel filters with 0.80 micron pores. The filters were dissolved in 10 ml of acetone and the solution analyzed without further treatment. The same equipment was used as for the gloves residues.

Surface extraction of leaves and fruit was performed on day of application and 2,4,8, and 26 days later for leaves and 2 and 4 days later for fruit. All fruit was harvested before the eighth post-application day. The fruit was extracted by placing it in wide-mouthed jars and adding benzene at the rate of 1 ml/4g of fruit. The jars were sealed and tumbled for 15 minutes. The benzene was decanted off and filtered through anhydrous sodium sulfate into screw cap bottles and refrigerated until analyzed by the above technique.

A leaf punch was used to remove a 14 cm^2 disc from the center each of 20 randomly selected leaves. The discs were placed in a four-ounce jar and extracted by shaking with 20 ml of benzene. The extract was treated as that of the fruit.

The study was supervised by Dr. J. Blair Bailey, Pesticide Safety Specialist for the University of California. Dr. Erwin P. Brauner, Chairman of the Public Health Committee of the Tulare County Medical Society, conferred with the investigating team and offered his cooperation.

Following is the data from the study.

Table I

CHARACTERISTICS OF PICKERS

PICKER	SEX	AGE	HEIGHT (INCHES)	WEIGHT (POUNDS)	YEARS PICKING	WORKED PAST MONTH	PREVIOUS TEST	OTHER DATA
1	M	24	65	150	4	Yes	No	a
2	M	44	65	135	12	Yes	No	b
3	F	34	64	126	10	Yes	No	
4	M	34	66	165	0	2 Wks	No	
5	M	21	66	120	5	Yes	No	c
6	M	45	66	130	5	Yes	No	
7	F	29	60	132	4	Yes	No	
8	M	24	64	139	2½	Yes	No	
9	M	31	69	186	8	Yes	Yes	
10	F	36	62	140	4	Yes	No	
11	F	17	63	107	4	Yes	No	
12	M	46	64	127	12	Yes	No	d
13	F	57	62	130	5	Yes	no	
14	M	72	65	140	40	Yes	No	e
15	F	16	62	110	3	Yes	No	
16	F	15	61	105	3	Yes	no	f
17	F	38	61	160	11	Yes	No	g
18	F	19	58	125	1	Yes	Yes	
19	F	19	60	100	4	Yes	No	
20	M	25	69	162	0	1 Wk.	No	
100	M	46	64	160	20	Yes	Yes	h
101	M	60	67	190	52	Yes	No	g

a. Under treatment for chronic headaches.
b. Diabetes controlled by oral medication.
c. Chronically poor appetite.
d. Has peptic ulcer, controlled with antacids.
e. Occasional low back pain.
f. Fractured skull 1969, no sequellae.
g. Anemia 1968, no current treatment.
h. Supervisor not intimately associated with picking.
i. Supervisor, crew foreman.

Table II

RESULTS OF CHOLINESTERASE TESTS

PICKER	7-24		7-25		7-27		7-31	
	RBC	PLASMA	RBC	PLASMA	RBC	PLASMA	RBC	PLASMA
	A. PICKERS WORKING IN W.P. - SPRAY BLOCK							
1	11.82	1.94	-	-	-	-	-	-
2	9.26	2.87	8.74	3.10	10.28	1.03	6.58	0.42
3	11.96	2.20	11.99	2.29	8.40	1.99	11.09	0.83
4	13.39	2.04	12.20	1.99	-	-	11.37	0.36
5	14.38	3.18	-	-	-	-	12.75	0.83
6	10.40	2.02	-	-	-	-	-	-
7	8.47	2.73	-	-	12.64	1.57	8.52	0.60
8	-	-	10.43	4.91	14.79	4.72	11.23	0.88
9	-	-	-	-	-	-	13.86	1.02
12	10.11	2.13	9.87	2.11	13.27	1.69	9.91	0.46
17	-	-	11.28	3.15	-	-	-	-
18	-	-	-	-	-	-	13.78	2.59
	B. PICKERS WORKING IN E.C. - SPRAY BLOCK							
10	12.67	3.25	-	-	QNS	-	12.54	2.91
11	12.39	2.80	-	-	14.86	2.80	12.13	2.59
13	12.25	2.37	-	-	15.00	2.69	-	-
14	11.53	1.76	-	-	12.71	1.74	-	-
15	12.82	2.30	-	-	8.68	2.22	12.27	1.99
16	11.96	2.75	-	-	-	-	11.78	2.45
20	-	-	-	-	-	-	13.93	2.26
	C. SUPERVISORS							
100	-	-	15.37	4.10	-	-	-	-
101	-	-	14.10	4.05	-	-	16.08	3.10

Normal Ranges for Titrimetric (pH Stat)
Method As Established By Laboratory:

Plasma	2.00 to 5.50 uM/ml/min (males)	1.20 to 4.00 (females)
Red Cells	8.00 to 17.00	7.50 to 15.50

MEANS OF RED BLOOD CELL CHOLINESTERASE ACTIVITY

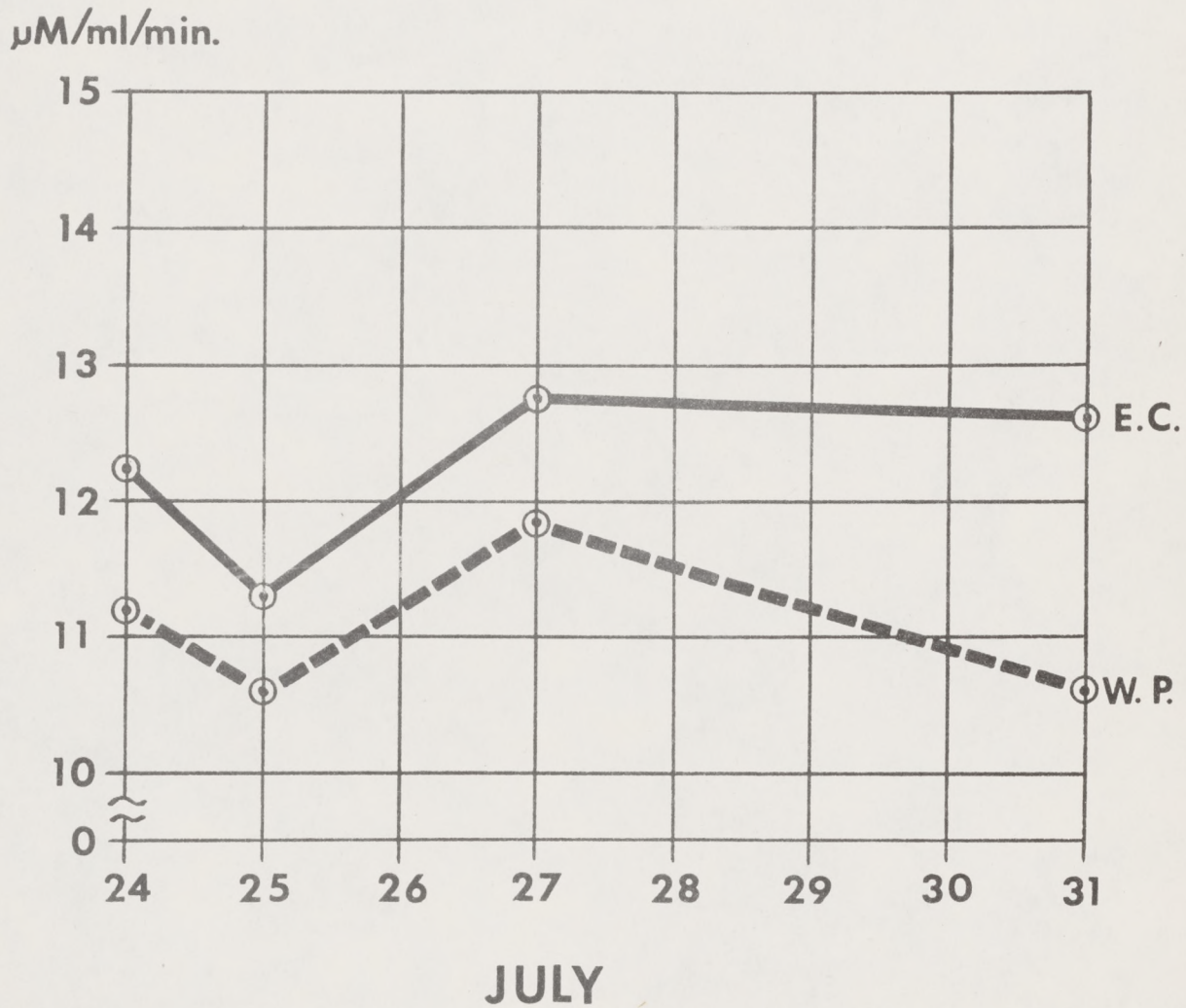

Fig. 1

MEANS OF PLASMA CHOLINESTERASE ACTIVITY

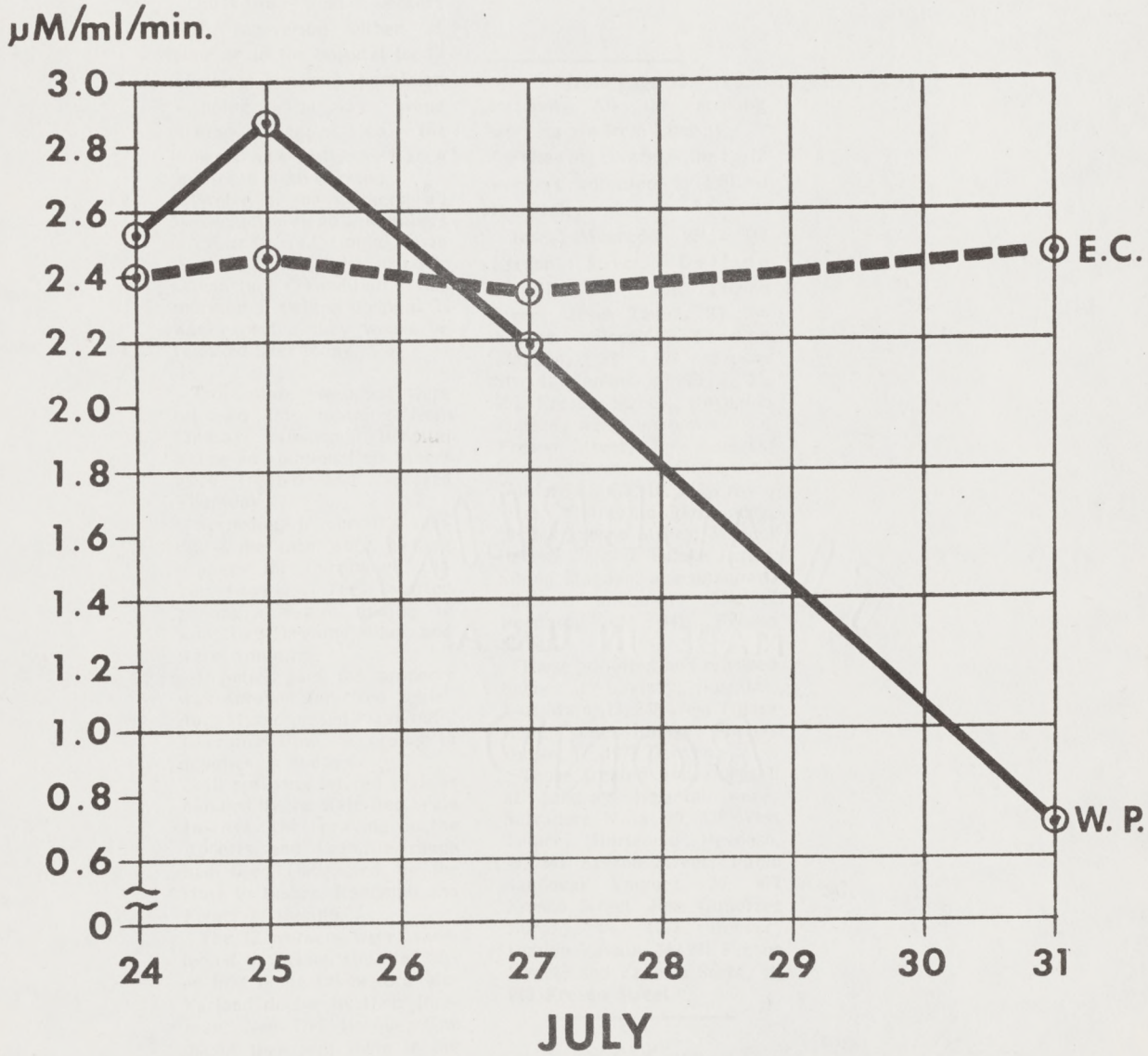

Fig. 2

Table III

AIR SAMPLES, BREATHING ZONE OF PICKERS
(Micrograms per cubic meter of air)

EQUIPMENT: Unico Micronair Personnel Air Sampler
SAMPLING PERIOD: 71 minutes in each case

SAMPLE	DATE	BLOCK	TEMPERATURE		HUMIDITY		WIND (MPH)	ETHION	MONOXON	TOTAL
			Start	Finish	Start	Finish				
1	7-28	general	-	90**	-	-	5-6	None detected		
2	7-29	WP	65	72* 79**	76	75* 72**	0-3	16.1	49.0	65.0
3	7-29	EC	65 65	72* 79**	76 76	75* 72**	0-3 0-3	8.3	38.7	47.0
4	7-30	WP	74 78	87* 96**	52 51	47* 43**	0-3	58.5	167.0	225.5
5	7-30	EC	74 78	87* 96**	52 51	47* 43**	0-3 0-3	5.0	23.2	28.2

* In Shade
** In Sun

Sample #1 Represents general air condition and was obtained from both blocks.

Sample #2 - 5 Represents breathing zone condition of picker in block indicated.

Check filters fortified with ethion in the laboratory demonstrated 88% and 102% recovery by extraction method used.

Table IV

ETHION EXTRACTED FROM PICKER' GLOVES

New cotton gloves were issued to ten pickers at start of work on 7-29-70. The gloves were recovered at end of work on 7-31-70

PICKER	µg ETHION* EXTRACTED/CM^2 GLOVE
	A. WP Block
4	128
7	73
8	64
5	Gloves not returned
12	94
	B. EC Block
10	14
11	15
13	18
14	13
15	15

* Includes ethion monoxon.

Table V

ETHION* RESIDUES REMAINING ON TREE SURFACES

DAYS AFTER APPLICATION	WP SPRAYED BLOCK	EC SPRAYED BLOCK
	A. Leaves	
0	1.87 $\mu g/cm^2$*	1.64 $\mu g/cm^2$*
2	1.12	1.09
4	1.01	0.76
8	0.67	0.56
26	0.21	0.20
	B. Fruits	
	PPM	PPM
0	-	-
2	1.14	1.25
4	1.31	1.50
8	All fruit picked	

* Includes ethion monoxon.

Note: Conversion to ppm may be accomplished by multiplying $\mu g/cm^2$ by 70.

SUMMARY AND CONCLUSIONS

Pickers, working 3 to 7 days after an orange grove was sprayed with 2 ethion formulations, were monitored by observation by a qualified physician, cholinesterase determinations, breathing zone air samples, and glove extractions. Surface residues of leaves were obtained 2, 4, 8, and 26 days after spraying.

The air samples indicated that picker exposure via inhalation was less than 0.25 mg per cubic meter of air in the case of wettable powder, and 0.05 mg per cubic meter of air for the emulsifiable concentration. These levels compare favorably with the ACGIC threshold limit values for other organophosphates (azinphos methyl - 0.2, dichlorvos - 1.0, Dibrom - 3.0).

Cholinesterase studies indicate that the vital erythrocyte cholinesterase was not affected by exposure during the course of the study. Plasma pseudocholinesterases were significantly depressed by exposure to the trees sprayed with wettable powder, but there is considerable doubt among toxicologists that this represents a significant biochemical lesion.

The glove study indicates that the pickers are presented with a dermal exposure which is much less when an emulsifiable concentrate is used.

The leaf and fruit surface residue studies indicate that approximately equal amounts of ethion are present when either a wettable powder or an emulsifiable concentrate is used. The lower air and glove concentrations in the workers exposed to emulsifiable concentrate would suggest that this formulation is bound more tightly to the surfaces.

The study in total demonstrates, we believe that pickers working in trees sprayed with ethion emulsifiable concentrate are subjected to no toxic exposures. Those working in trees sprayed with wettable powder are probably not either, but the significance of depression of plasma pseudocholinesterases is not universally agreed upon.

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Anderson
analysis
of
Niagara

FORM 7 (REV. 3-68)

MESSAGE

To_____

Date_____ Time_____ A.M. P.M.

WHILE YOU WERE OUT

Miss
Mrs.
Mr._____

Of_____

Phone_____

Phoned	☐	Was in	☐
Please call	☐	Will call again	☐
Will phone again . . .	☐	Would like to see you .	☐
Returned your call . . .	☐	No message	☐

Message_____

_____

_____

_____

_____ _____

*Received by*_____

Have you made a suggestion today?
Send your ideas to Merit Award Board

Comments on Niagara Chemical's cholinesterase studies of orange pickers exposed to ethion:

First, and most basically, the research design was such that it could not and did not test the company's starting hypothesis: namely, that a thirty-day waiting period for ethion is longer than necessary. Or, to put it another way, the question "What is the 'no effect' interval between application and re-entry?" -- which was the point of the Department of Agriculture's emergency order of June 19, 1970, and was supposed to be the point of Niagara's study -- remains unanswered.

Reduced to its essentials, the company's research design was to test workers twice "before exposure," once following an interval of three days from application and, finally, after an interval of seven days from application. However, for practical purposes, the third of these four blood tests should be considered part of the "before exposure" series since the samples were drawn at approximately 9:30 in the morning of the first day of work. In other words there was only one "after exposure" test -- seven days after ethion was applied to the grove, and four days after picking commenced. This paucity of "after exposure" information makes it impossible to construct a meaningful time series and is virtually fatal to a study which purports to be longitudinal in character.

Another grave limitation of this study is the small number of workers tested. At one time or another, twelve individuals took part in the portion of the study having to do with ethion applied in the form of wettable powder; seven individuals were involved, at one time or another, in the other half of the study dealing with ethion applied in the form of emulsifiable concentrate. there is a serious question whether numbers of this magnitude would have been sufficient to permit statistically stable conclusions, even if all subjects had taken part in all four blood cholinesterase tests. But they did not. In the wettable-powder group, for example, subjects 1 and 6 were tested on the first day but not on any of the three subsequent occasions; subject 17 took part only in the second test; subjects 9 and 18 took part only in the fourth test. Yet all these data were included in the company's final calculations just as though these subjects had participated throughout the study. When such improper cases are eliminated, the results are quite different.

Before proceeding to an examination of the cases which may legitimately be included in a longitudinal study, some of our methodological assumptions should be noted. As implied a moment ago, the first three serological tests may all be thought of as "pre-exposure." They should be viewed as a whole, a series which takes on valid meaning only when each of the parts is considered in the light of the other two. It bears no resemblance to reality to assume (as Niagara analysts assume) that the first in the series is "normal" or a "base line" for the individual involved. The first samples were drawn on July 24, a Friday, after the subjects had been working all week in various orange groves. Niagara analysts made no effort to ascertain what these groves were, what organophosphates may have been applied to them or when -- although this information was easily available from the employer, the Euclid Citrus Association of Lindsay.

There is every reason to believe, from the internal evidence of this study as well as from everything observed by our Community Studies on Pesticides in its research among Tulare County citrus workers, that the cholinesterase levels of these subjects were in a highly dynamic rather than a "normal" state. Some were descending as the result of recent subacute exposures; others, whose exposure may have been in the more distant past, were ascending.

Under these circumstances, the closest possible approximation to an individual's true "base line" is to take the highest of the pre-exposure readings. Organic phosphates, and perhaps other factors, may depress cholinesterase values; but nothing, so far as we know, can "artificially" elevate an individual's level above his true normal.

Given the design of this study, it is necessary to stress that the best available pre-exposure values -- i.e., the highest -- are very crude indicators. The length of time between the first pre-exposure test and the third -- from Friday, July 24, to Monday July 27 -- was not nearly enough for either plasma or erythrocyte cholinesterase activity to find its true level. If real base line data had been obtainable, they would no doubt have been substantially higher than anything which appears in the following analysis, and the changes observed after exposure would have been proportionately greater.

Let us now proceed to examine the results when the above methodological assumptions are applied to each of the four major classes of data: RBC cholinesterase values, emulsifiable concentrate; plasma cholinesterase values, emulsifiable concentrate; plasma cholinesterase, wettable powder; and RBC cholinesterase, wettable powder.

Table I

ERYTHROCYTE CHOLINESTERASE VALUES, PICKERS IN NAVEL ORANGE GROVE SPRAYED WITH EMULSIFIABLE CONCENTRATE ETHION, 7 POUNDS/ACRE

CASE	BEST "PRE-EXPOSURE" MEASUREMENT	"POST-EXPOSURE" MEASUREMENT[1]	CHANGE	PERCENT CHANGE
10	12.67*	12.54	- .13	- 1.0
11	14.86**	12.13	-2.73	-18.4
13		Insufficient data		
14		Insufficient data		
15	12.82**	12.27	- .55	- 4.3
16	11.96*	11.78	- .18	- 1.5
20		Insufficient data		
Mean	13.01	12.18	- .83	- 6.4

[1] Seven days after application of material; four days after re-entry into grove.

* Based on single test.

** Based on two tests.

As may be seen, not one of these seven cases met the criterion we believe minimal: three pre-exposure tests. Two had had two pre-exposure blood samples; two had had only one; three had either had none at all, or were missing the equally crucial post-exposure test.

Because of the variation in the degree of change of cholinesterase activity--from -1.0% to -18.4% -- statistical tests do not permit any stable conclusions to be drawn from these four cases. It is manifestly impermissible for the company to assert that "pickers working in trees sprayed with ethion emulsifiable concentrate are subjected to no toxic exposures". By the same token, if the decision is based on erythrocyte values alone (plasma levels are quite another matter, as will be seen in a moment), it would be statistically presumptuous to assert that ethion emulsifiable concentrate does affect RBC cholinesterase significantly. But if one is interested in data which are suggestive, if not conclusive, it is surely suggestive that all the findings point in the same direction: a downward drift after exposure. Larger numbers of subjects and more examinations of each subject would demonstrate whether this drift is a statistical artifact or whether it is real.

Table II

PLASMA CHOLINESTERASE VALUES, PICKERS IN NAVEL ORANGE GROVE
SPRAYED WITH EMULSIFIABLE CONCENTRATE
ETHION, 7 POUNDS/ACRE

CASE	BEST "PRE-EXPOSURE" MEASUREMENT	"POST-EXPOSURE" MEASUREMENT[1]	CHANGE	PERCENT CHANGE
10	3.25*	2.91	-.34	-10.5
11	2.80**	2.59	-.21	- 7.5
13	Insufficient data			
14	Insufficient data			
15	2.30**	1.99	-.31	-13.5
16	2.75*	2.45	-.30	-10.9
20	Insufficient data			
Mean	2.78	2.48	-.30	-10.6

1 Seven days after application of material; four days after re-entry into grove.

* Based on single test.

** Based on two tests.

Although the numbers of usable cases in Table II are as small as those in Table I, the degree as well as the direction of the observed change is so consistent that the null hypothesis may be tested by the following formula:

$$t = \frac{\bar{X}_d - \mu_d}{\sqrt{\frac{\sum (X_d - \bar{X}_d)^2}{N}} \Big/ \sqrt{N - 1}}$$

$\bar{X}_d$ = average change (-10.6%)

μ_d = null hypothesis -- i.e., no change (0.0%)

$X_d - \bar{X}_d$ = difference between observed changes and average change. (The number under the larger radical sign -- i.e., the sum of the squares of the differences divided by the number of cases -- represents the sample standard deviation of differences)

$N - 1$ = degrees of freedom (i.e., number of changes observed, minus one)

This is the well-known "t test" or "Student" test. Applied to the data in Table II, it yields a "t" of 8.626. In level-of-significance tables which may be found in any standard statistical textbook, a "t" of this magnitude, in a two-tailed test with three degrees of freedom, proves to be significant at the 1% level of confidence.

In other words, the impact of ethion emulsifiable concentrate on plasma cholinesterase is very significant indeed. If Niagara had submitted its data to any kind of statistical analysis, rather than merely to gross graphical presentation, it could not have concluded that ethion in emulsifiable concentrate form is non-toxic.

Table III

PLASMA CHOLINESTERASE VALUES, PICKERS IN NAVEL ORANGE GROVE
SPRAYED WITH ETHION WETTABLE POWDER
7 POUNDS/ACRE

CASE	BEST "PRE-EXPOSURE" MEASUREMENT	"POST-EXPOSURE" MEASUREMENT[1]	CHANGE	PERCENT CHANGE
1		Insufficient data		
2	3.10	0.42	-2.68	-86.5
3	2.29	0.83	-1.46	-63.8
4	2.04*	0.36	-1.68	-82.4
5	3.18**	0.83	-2.35	-73.9
6		Insufficient data		
7	2.73*	0.60	-2.13	-78.0
8	4.91*	0.88	-4.03	-82.1
9		Insufficient data		
12	2.13	0.46	-1.67	-78.4
17		Insufficient data		
18		Insufficient data		
Mean	2.91	0.63	-2.28	-78.4

[1] Seven days after application of material; four days after re-entry into grove.

* Based on two tests.

** Based on single test.

From Table III, it may be seen that the numbers of usable cases in the wettable powder study were slightly greater than in the emulsifiable concentrate study. Three cases met the criterion of three pre-exposure tests; three subjects received two pre-exposure tests; one had only one.

The changes are so overwhelming that even with such small numbers, firm conclusions are possible. Niagara Chemical analysts admit that "plasma pseudocholinesterases were significantly depressed by exposure to the trees sprayed with wettable powder". But the conclusion they draw from this fact is not one with which many public health authorities would agree. The company argues that the physiological function of plasma cholinesterase has not been proven in the

same way as that of red blood cell cholinesterase, and therefore its depression is not clinically significant.

That may be true, but the statistical significance of plasma cholinesterase inhibition in the ranges observed here -- from 64% to 85% -- is beyond the doubt of reasonable men. Such changes are "associated in time" with the onset of lassitude, dizziness and the other early indicators of organic phosphate poisoning. They may not be causally related but they serve as a flag, a warning, that unless the individual is removed from the exposure, more overt symptoms -- "significant biochemical lesions", as the company puts it -- are likely to follow.

In these terms, wettable powder ethion must be considered a very hazardous substance indeed, even after a waiting period of seven days, which is seven days longer than the waiting period to which the company proposes to revert. How much beyond seven days the "no effect" period lies, of course, one cannot say on the basis of a study which lasted only seven days.

Table IV

ERYTHROCYTE CHOLINESTERASE VALUES, PICKERS IN NAVEL ORANGE GROVE SPRAYED WITH WETTABLE POWDER ETHION, 7 POUNDS/ACRE

CASE	BEST "PRE-EXPOSURE" MEASUREMENT	POST-EXPOSURE" MEASUREMENT[1]	CHANGE	PERCENT CHANGE
1		Insufficient data		
2	10.28	6.58	-3.70	-36.0
3	11.99	11.09	- .90	- 7.5
4	13.39*	11.37	-2.02	-15.1
5	14.38**	12.75	-1.63	-11.3
6		Insufficient data		
7	12.64*	8.52	-4.12	-32.6
8	14.79*	11.23	-3.56	-24.1
9		Insufficient data		
12	13.27	9.91	-3.36	-25.3
17		Insufficient data		
18		Insufficient data		
Mean	12.96	10.21	-2.75	-21.2

[1] Seven days after application of material; four days after re-entry into grove.

* Based on two tests.

** Based on single test.

Niagara Chemical chooses to rest its case principally upon the data in Table IV. It is therefore appropriate to examine them with special care. In the first place, it may be noted that subject 5 received only one pre-exposure examination. He should probably be eliminated from the series, along with subjects 1, 6, 9, 17 and 18, on grounds of inadequate information. If this is done, the mean before exposure becomes 12.73, after exposure, 9.78 and the change for the whole series shifts from -21.2% to -23.2%. It is difficult to reconcile these findings with the company's claim that "the vital erythrocyte cholinesterase was not affected by exposure during the course of the study".

Perhaps the company will argue that the figure of -23.2% is less than the rule of thumb which is sometimes used in RBC cholinesterase studies: up to 25% variation may be accepted; only depressions of 25% or more are considered significant enough to warrant medical intervention or surveillance. The rule of thumb, however, like all rules of thumb in clinical medicine, was devised with individual cases in mind. (Even on that basis, it will be noted that three subjects were "over the limit" and another practically so).

The entire purpose of statistical research, as distinguished from clinical medicine, is to allow random individual variations to cancel one another out, thus permitting ever more precise statements as the numbers of cases grow larger. Powerful, impeccable statistical tests are available which enable the researcher to say, within far narrower limits than 25%, whether the changes he observes are significant or not. It is not apparent, from this report, that Niagara's research department is familiar with these statistical tools.

In the present case, the statistical test of choice is the "t-test", or "Student test", already discussed. Applying the same formula to the changes observed in Table IV yields a "t" of 5.247. In a distribution with six degrees of freedom, this is significant well beyond the 1% level. Only once in six or seven hundred trials would changes of this magnitude and consistency be expected to result from chance factors.

In short, when submitted to universally accepted statistical techniques, Niagara's own data disprove its contentions. Ethion in the form of emulsifiable concentrate has a highly significant effect on plasma cholinesterase; more data are needed to evaluate conclusively its effect on RBC cholinesterase. Ethion in the form of wettable powder has a highly significant effect upon both plasma and RBC cholinesterase.

The company's request to return to the status quo ante (i.e., "may be applied up to the day of harvest") must therefore obviously be rejected. Ethion is highly toxic to orange pickers seven days after application. How much beyond seven days it remains toxic, no one could say from the data presently in hand. The burden rests with the company. Unless and until it generates statistically acceptable data proving that a 14-day waiting period is safe, or 21 days, or some other period, there is no basis on which to alter the present 30-day interval between application and re-entry.

Statistical conservatism, not to mention worker health conservation, dictates a continuation of the state Director Agriculture's regulations of June 19, given the present state of knowledge.

The data of Niagara, relative to the rate of loss of ethion from citrus leaves, was subjected to kinetic analysis. It must be considered only as a first approximation since the number of post-application leaf samples - five - is too limited to allow more critical considerations.

Data were plotted with the assumption that pesticide loss would follow first order kinetics. As is shown in Figure 3, this was a reasonable assumption. Following an initial rapid breakdown or loss of some 40% of the pesticide, over the first 2 days, there appeared to be a first order decay. For the wettable powder, between the second and twenty sixth days, the specific rate constant approximated - 4.8×10^{-2} days $^{-1}$ with a half life of about 10 days. Reckoning from day 2, some 37% of the original material is still available by day 14 while 22% remains after 21 days. Considering the extensive loss in cholinesterase activity, particularly with the plasma enzyme, one might conjecture that a waiting period of at least 23 days post-application should be considered.

A number of physical factors may well affect the rapid initial loss - photochemical oxidation of material on the outer leaves (followed by the slower first order loss of material residual on the inner surfaces), loss by wind erosion, evaporation, washing or more extensive hydrolysis by dew which settles more heavily on the outer surfaces, evaporation at the higher daytime temperatures incumbent on outer leaves, etc. Because citrus pickers are in intimate contact with foliage it is more appropriate to view the data for safety purposes, in the light of the results obtained from day 2 on.

There is yet another item bearing on any assessment of hazards based on residue data. The method by which residual pesticide is removed from leaves - particularly citrus which support a heavy cuticle - bears heavily on the amount which will be recovered. If the method accounts for the removal of only the material physically bound to the leaf surface, less, obviously, will be recovered than if the leaves were washed in a solvent which removed all available pesticide <u>on</u> the cuticular leaf surface. In turn, greater amounts will be recovered if the leaves are treated with a solvent which also removes the cuticle and any dissolved pesticide. Still more may be recovered if leaves are ground with a solvent for the pesticide. Such was the case with Niagara, who ground leaves in benzene.

Thus, the levels of residual pesticide may be unduly high, by analysis, insofar as worker safety is concerned. On the other hand, not much information is available suggesting the degree of removal of residue from leaf cuticle by abrasive contact with human skin.

Because the number of leaf samples taken was so small, and because the distribution of points representing concentration ratios in the kinetic plot for the emulsifiable concentrate (Fig. 3) was such as to allow only three per lineal representation, no further analysis was made. As is noticed, the rates of loss may be less than for the wettable powder, but only slightly so. A diminished loss is to be expected since the

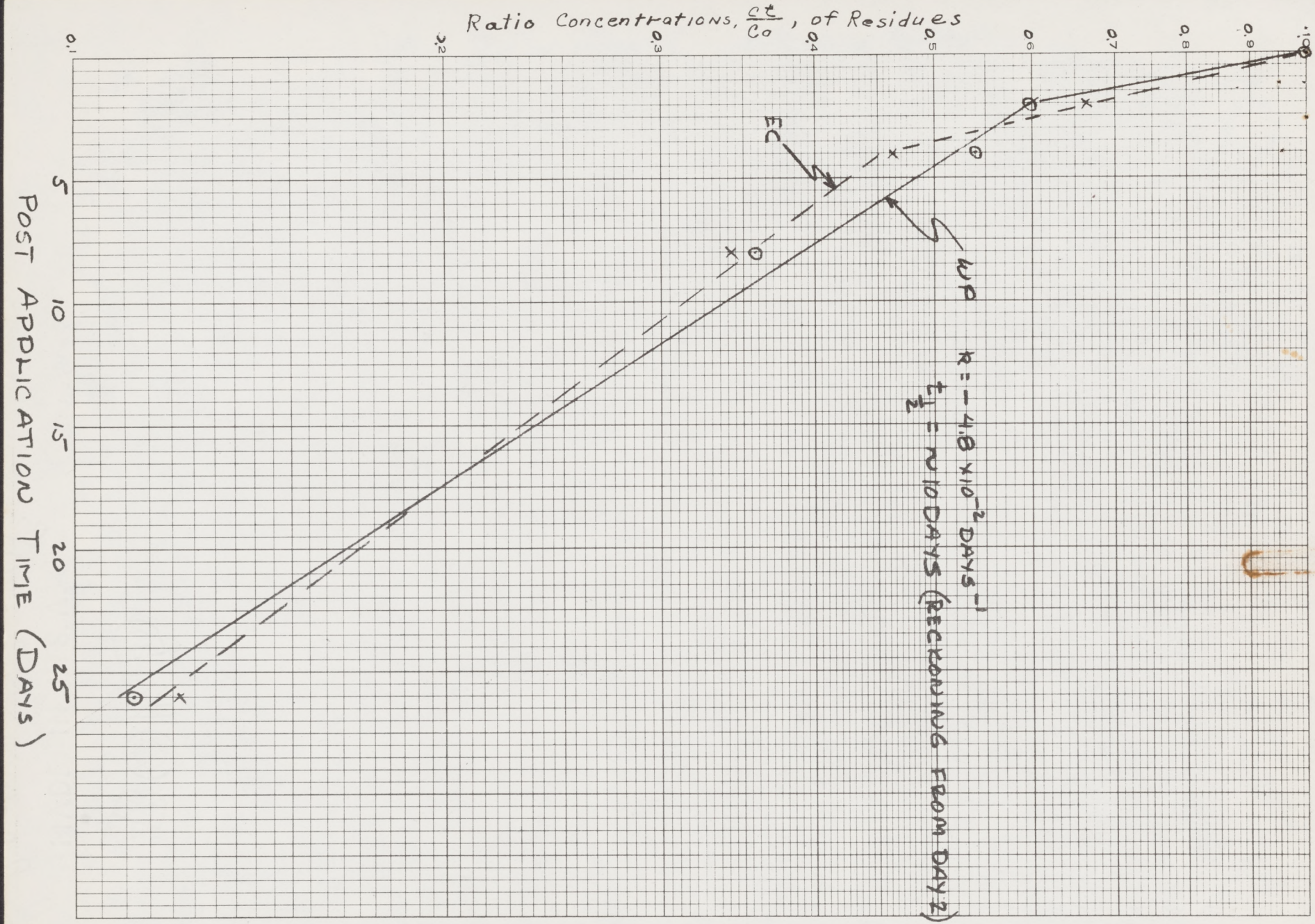

Fig. 3. Rate of decay of ethion, wettable powder and emulsifiable concentrate on citrus leaves. Data from Niagara Chemical Division, FMC. Co and Ct refer to concentrations at the time of application and at time t, respectively.

emulsifiable concentrate, applied in a solvent, should adhere to the cuticle more strongly than the powdered preparation. It was surprising that the initial loss, over the first 2 days was somewhat greater than for the powder but with a paucity of applicable points on the plot, this effect may be purely an artifact.

The cholinesterase activity data reported above suggest that at least some of the pesticide applied as an emulsifiable concentrate is available for absorption by human skin.

Two additional sets of data regarding the loss of ethion residues will appear on the pages that follow. There was a similarity in the data depicting ethion loss as measured by either the Chemagro Corporation or the California Department of Agriculture. Thus, only one other kinetic analysis for ethion has been done - reviewing the data of Agriculture.

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Chemagro Report No. 28250
October 7, 1970

DETERMINATION OF THE HAZARDS TO WORKERS PICKING CITRUS TREATED WITH GUTHION WETTABLE POWDER FORMULATION

(Report of Chemagro Corporation)

I. Introduction

Treatment of citrus groves with GUTHION has been a practice in California for many years. The pre-harvest interval on the Federal label is 7 days. This interval has not resulted in any toxicity problems for workers in the past. However, in May, 1970, the California Department of Public Health investigated an incident in Tulare County, California, where 16 orange pickers became ill.

It was known that the citrus grove where the workers were picking had previously been treated with parathion, ethion and GUTHION. It was also known that the workers had been previously exposed to delnave, dimethoate and probably other compounds.

As a result of the incident in Tulare County, the California Department of Agriculture and the California Department of Public Health increased the 7-day pre-harvest interval to 30 days. In view of the fact that the workers involved in the Tulare County incident had been exposed to a number of pesticides, we decided to carry out further tests to see if a 30-day interval for GUTHION was really necessary. The test protocol that was developed provided for cholinesterase tests for workers and collection of residue data for fruit, foliage, gloves, skin patches, urine and air. They also requested residue data to show what effect other pesticides had on residues of GUTHION and vice versa. Since GUTHION and ethion are most frequently used in combination on citrus, ethion was chosen as the other pesticide used in our study.

Briefly, the test consisted of treating a block of orange trees with GUTHION and several smaller areas with GUTHION and ethion to get a side-by-side comparison of residues. The Wettable Powder (WP) formulations were used because it is believed that the greatest hazard to workers may be due to inhalation of dust containing the pesticide. The WP should give the maximum inhalation exposure. The rate of GUTHION used according to the Federal Label is as high as 2,000 gallons of the spray mixture per acre. Since the normal use rate is 1,000 gallons per acre of spray mixture containing 6 ounces Guthion active per 100 gallons, this rate was used.

Workers entered the main block on the seventh day after treatment. Plasma and erythrocyte cholinesterase baselines were established for each worker one week prior to their entering the treated block, and their cholinesterase levels were measured on the second and fifth day after they picked fruit in the treated block. The test was to cover a period of ten days of picking. If there was an effect, the assumption was that it would appear within the first ten days.

Throughout the test, residue samples of leaves and fruit were collected from the main block and smaller areas. Surface residues on leaves and fruit were determined by washing with water followed by benzene. Residues readily

removed by water should present the greatest potential hazard to workers, since they of fer the greatest possibilities to dermal and inhalation exposure. Gloves, skin patches and air samples were also collected to obtain a measure of exposure.

The details of the procedures are now described.

II. Experimental

Before the study was started, a protocol was written and sent to the State of California for their approval and suggestions. This protocol has been followed throughout the study.

Orange Grove

A 25-acre grove oranges which had not been sprayed with cholinesterase inhibiting pesticides for at least 30 days was located in the Terra Bella Orchard, Visalia, California. The trees showed an average cover of dust. The grove was divided and the first part (Block 1) was sprayed with GUTHION WP, 6 ounces active per 100 gallons, 1,000 gallons per acre on August 24, 1970. Block 2 was sprayed on August 31, 1970, 7 days after Block 1. The treatments of Blocks 1 and 2 were identical in every way.

Four additional areas of 8 trees each were treated with the following formulations:

AREA	FORMULATION OUNCES ACTIVE/100 GAL.	VOLUME GAL./ACRE
A	GUTHION Emulsifiable Concentrate (EC) - 6	1000
B	Ethion WP -	1000
C	GUTHION WP - 6 Ethion WP - 6	1000
D	GUTHION WP - 6	2000

Four areas were treated at the same time as Block 1, and four areas were treated at the same time as Block 2.

Collection of Residue Samples

Pre-treatment samples of leaves and fruit were taken from Blocks 1 and 2 for residue analysis. Samples of leaves were taken from Block 1 at 0, 1, 3, 7, 9, 11, 15, 23 and 30 days after the spraying. Samples of leaves were taken from Block 2 at 0, 2, 4, 6, 8, 13, 15, 20 and 27 days after spraying. Four average size leaves were collected from each quadrant of each of 8 trees for the first 6 intervals of Block 1 and the first 4 intervals of Block 2. For the later intervals on each block, the number of trees sampled

was increased to 24 to get a better sampling. The leaves were collected so that the entire sample was comprised of a representative number from the interior and exterior portions of the tree.

Five pounds of fruit were collected at the same time in the same manner as the leaves. An effort was made to obtain representative samples from the surface and the interior of the tree to ensure an adequate sample. Care was taken to avoid loss of surface residue in the form of dust. Therefore, the fruit and leaves were each put into plastic bags and then put into a cloth residue bag.

Samples of leaves and fruit were also collected from the corresponding areas, A, B, C and D. Only a zero-day sample was taken from Area D to show if residues were higher due to the 2,000 gallon per acre volume as compared to the 1,000 gallon per acre.

For the first 5 intervals from Blocks 1 and 2 and their corresponding areas, leaves from the outer and interior portion of the tree were kept separate and analyzed separately to observe any differences in residues. For the remaining intervals the leaves were combined.

Workers

Workers of age 21 years or older who had no recent exposure to cholinesterase inhibiting pesticides were chosen. Baseline cholinesterase levels were established for 15 workers. Blood and urine samples were taken from each worker 7, 5 and 3 days prior to the start of the test. Workers whose cholinesterase levels were not within the normal range would have been eliminated from the test. Maria Montez had one plasma 1.57, and one erythrocyte value, 16.40, and Miguel Ortiga had one plasma value, 0.72, outside the normal range. They were allowed in the test, because the other two pre-treatment values were normal. During the time the baseline values were being established, the workers were not allowed to work in orchards, groves, etc. which had been sprayed with cholinesterase-inhibiting pesticides.

Starting the first day of picking, an attempt to collect a 24-hour urine sample from each worker was made. Workers were supplied with urine collection bottles. An aliquot was taken and shipped to Kansas City for analysis.

The blood samples were taken at the end of a full day of labor on the eighth and eleventh day after spraying Block 1. Blood samples were collected by venipuncture. The analytical method was the pH stat assay for human blood cholinesterase recommended by the United States Public Health Service. The analytical values reported are pH stat unit values (μ mol acetylcholine/min/ml). Three pre-exposure blood samples were collected over a 7-day period. The average of these values was calculated as the normal mean value for this particular individual. The values for the individual, after he started to work in the treated grove, were compared to his normal mean value to measure effect.

Starting the first day of picking, two workers were chosen to wear new cotton gloves, skin patches, one on the forearm and one on the head, and to carry an air sampling device, a Telmatic 150 Air Sampler. The exact time for wearing the gloves, patches and air sampling device was sixty minutes. The following day and on each successive day, two new workers wore fresh gloves and patches and carried the air sampling device. The gloves, patches and mineral oil from the air sampler trap were sent to Kansas City for analysis. On each successive day the time which the workers wore the gloves, patches and air sampler was an hour later in the day to observe any differences in residues as the day progressed. The remaining workers in the test wore their own gloves while they picked fruit.

The air flow in the air sampling device was 3.0 liters per minute, and was collected near the worker's face. Each gauze patch was 5.25 square inches.

All the orange pickers spent from six to seven hours in the grove each day. Only G. Salas spent less time in the grove, four to five hours per day. Two workers listed in Table VI did not pick oranges, E. Johnson, citrus packer representative, and L. Martinez, labor contractor.

Weather Data

The weather data were recorded in the Monthly Climatic Summary, Lindcove, California, a field station of the University of California and are summarized in Appendix 1. There was no rainfall throughout the study.

Analysis of Residue Samples - Procedure

Leaves:

1) The leaves and bag were weighed.

2) The bag was cut into 4 pieces and rinsed thoroughly in 400 ml. water, followed by a second rinse in a second 400 ml. water.

3) The leaves were rinsed in the same manner in bunches of 20-30 leaves, using the same water that was used for the bag rinses.

4) Contact time in all water rinses was 10 - 20 seconds.

5) The two beakers of water from the bag and leaf rinses were combined and adjusted to 800 ml. A 200-ml aliquot was extracted with 200 ml. chloroform.

6) One hundred milliliters of the choloroform extract from the leaves and bag was evaporated to dryness and the residue was re-dissolved in 2 ml. acetone.

7) The leaves were then washed in two 400 ml. portions of benzene.

8) The benzene extracts were combined and diluted to 800 ml.

A 100 ml. aliquot was evaporated to dryness and the residue was dissolved in 2 ml. acetone

Oranges:

1) The oranges and bag were weighed.

2) The bag was treated in the same manner as above.

3) Each orange was stabbed with a micro spatula with a sharp point and swirled ten times in 400 ml. of water, and then in a second 400 ml. of water. Contact time in each water wash was 5 - 10 seconds.

4) Step 3 was repeated for all oranges in the sample.

5) The two water extracts were combined and diluted to 800 ml.

6) A 200 ml. aliquot was extracted with 200 ml. chloroform. The chloroform was evaporated to dryness, and the residue was dissolved in 2 ml. acetone.

7) The oranges were then rinsed in two 400 ml. portions of benzene in the same manner as in Step 3.

8) The benzene extracts were combined and diluted to 800 ml. A 100 ml. aliquot was evaporated to dryness and the residue was re-dissolved in 2 ml. acetone.

Gloves:

1) Each pair was immersed and mixed in 400 ml. chloroform for 3 - 5 minutes.

2) The chloroform was squeezed from the gloves.

3) A 100 ml. aliquot was evaporated to dryness and the residue was re-dissolved in 2 ml. acetone.

Mineral Oil:

1) The contents (30 ml.) of the trap from the air sampling unit were emptied and the trap was rinsed twice with 5 - 10 ml. mineral oil. The rinses were combined with the original contents and shipped in a stoppered glass jar to Kansas City.

2) The contents of the jar were rinsed into a separatory funnel, using 200 ml. Skellysolve B.

3) The Skellysolve B was extracted with 100 ml. acetonitrile.

4) A 50-ml. aliquot of acetonitrile was evaporated to dryness and

the residue was re-dissolved in 2 ml. acetone.

5) The Skellysolve B extract was placed in a tared, round-bottomed flask.
6) All the Skellysolve B was evaporated, and the weight of the mineral oil was determined by difference in flask weight.

Patches:

1) The patch (gauze) was placed in a 4-ounce bottle with 50 ml. chloroform.

2) The bottle was stoppered and shaken for five minutes.

3) A 25 ml. aliquot was evaporated to dryness and the residue was re-dissolved in 2 ml. acetone.

Gas Chromatographic Procedure

Column: 14 inches by 2mm. i.d. glass, packed with 5% D.C. 200 on 70 - 80 mesh Chromosorb G

Temperature: Oven - 220°C
Inlet - 270°C
Detector - 290°C

Carrier Gas: Helium, 60 m./minute. 40 psig.

Instrument: Hewlett-Packard Model 5750, dual flame instrument modified for thermionic flame operation.

Four microliters of each acetone solution were injected and compared to a composite standard of GUTHION (6.25 microgram/ml.) and ethion (2.5 microgram/ml.). Acetone solutions containing high residues were diluted and compared to injected standards.

Calculations

Parts per million (ppm.) for both GUTHION and ethion were calculated from the general equation:

$$ppm. = \frac{\text{Sample Area}}{\text{Standard Area}} \times \frac{\text{Nanograms Standard Inj.}}{\text{Wt. of Sample (g.)}} \times \frac{\text{Final Volume (ml.)}}{\text{Microliters Inj.}} \times \text{Aliquot Fraction}$$

III. Results and Discussion

The results of the cholinesterase determinations are summarized in Table VI . The pre-exposure samples were taken on August 24, 26 and 28 for all workers except Jose Ramirez, George Gutierrez, Miguel Ortiga and Carlos Ortiga. Blood samples were taken from these workers on August 28,

29, and 31, immediately before they went into the treated orange grove. Exposure blood samples were drawn on September 1 and 4.

The post-treatment cholinesterase values were lower than the pre-treatment averages. The major effect appeared to be in the plasma values. It also appears that there was greater depression among the female workers (-52.83% plasma, -16.13% erythrocytes) as compared to male workers (-31.77% plasma, -13.45% erythrocytes).

A summary of the exposure of the workers is shown in Table VII. There appears to be no correlation between the residues from gloves, patches and by inhalation with their changes in cholinesterase levels. The poor correlation may be due to the fact that the exposures were measured for a short period of time and may not be a representative sample.

Urine samples have not yet been analyzed. A fluorometric method is currently being developed.

Surface residues of GUTHION were mostly removed from leaves with water, but not from fruit, Table VIII, where the values represent averages of several samples. This showed that residues would more likely wash off the leaves than the fruit. The residues on the fruit required a benzene wash for their removal. This indicates that residues on fruit may penetrate somewhat and that handling might not result in much removal of surface residue. The residue bags from leaves and fruit showed that approximately 2% - 3% was removed from the surface during shipment. This was also true for the ethion residues. A comparison of ethion and GUTHION average residues as a result of water and benzene washing is shown in Table IX. The results indicate that GUTHION was more readily removed from leaves by water washing than ethion. For fruit, there was little difference from water washing.

The residues of GUTHION in Blocks 1 and 2 and the GUTHION and ethion in the corresponding areas are shown in Figures 4 through 13. The residues in the leaves are read from the scale on the left, and the residues of fruit are read from the scale on the right. There was little difference in the residues on the leaves, whether the leaf was from the interior or surface of the tree.

Residues for leaves and fruit for the 2,000 gallon per acre rate were not significantly different at zero days from the 1,000 gallon per acre rate.

The residues of GUTHION were variable possibly because of not being able to collect a large enough sample. However, the residues of ethion were collected in the same way, and a steady decline of ethion residues is observed in every case.

In Block 1, Figure 4 , the 9-day interval appears to be out of line, and if this is true, then a gradual decline of residues is observed for the leaves. The fruit does not show as steady a decline. In Figure 5 , a gradual decline of GUTHION residues is seen in spite of the variability among the intervals. The results in Figures 7 and 8 indicate that GUTHION residues

are not affected by the presence of ethion.

IV. Summary

Orange trees were treated with GUTHION WP at the rate of 6 ounces active per 100 gallons and 1,000 gallons per acre. Smaller areas of trees were treated at the same rate with (1) GUTHION SC, (2) ethion WP, (3) GUTHION WP and ethion WP, and (4) GUTHION WP but with 2,000 gallon per acre. Surface residues on leaves and fruit were determined by first washing with water and then with benzene. Residues removed with water are presumably the greatest potential hazard to workers. The remainder of the residue was removed with benzene.

Baseline cholinesterase values from plasma and erythrocytes were established for 15 workers. The workers entered the main block of trees treated with GUTHION WP 6 ounces per 100 gallons and 1,000 gallons per acre. Cholinesterase levels were determined on the second and fifth day after picking.

Residues from GUTHION WP and SC showed a gradual decline, but residues were highly variable from one interval to the next. In the main block where workers were picking, the initial residues from GUTHION WP were approximately 100 ppm. on leaves and 0.6 ppm on fruit. After 30 days, the residues were approximately 30 ppm. on leaves and 0.40 ppm. on fruit. Residues of GUTHION were not affected by the presence of ethion residues.

The average cholinesterase levels of workers after five days of picking were -31.7% plasma and -13.5% erythrocytes for male workers and -52.8% and -16.1% for female workers. The depression of erythrocyte levels was less than 25% which is considered the significant level of depression. The depression of plasma levels was significant, but a correlation of the depression with residues on patches and in the air was not established. This could be due to limited data from workers wearing gloves and patches and collecting samples of air.

The average residues in air were approximately 0.1 micrograms per liter - one sample was 0.35 micrograms per liter. Residues in air seemed low, but a continuous exposure of workers to 0.1 microgram per liter might account for depression of plasma cholinesterase levels.

A "no-effect" level of cholinesterase depression from inhalation has not been determined for humans, but would be helpful in evaluation of hazards due to inhalation.

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Chemagro Report No. 28250
October 7, 1970

Table VI

PLASMA AND ERYTHROCYTE CHOLINESTERASE VALUES FROM WORKERS IN GUTHION - CITRUS STUDY

PARTICIPANTS' INITIALS	PRE-EXPOSURE SAMPLES			AVERAGE	POST-EXPOSURE SAMPLES		AVERAGE	DIFFERENCE	PERCENT DIFFERENCE
	1	2	3		1	2			
EJ									
Plasma	4.12	4.41	6.00	4.84	3.97	3.59	3.78	- .96	-19.8
Erythrocyte	15.88	13.37	14.82	14.69	13.60	12.99	13.30	-1.39	- 9.5
GS									
Plasma	3.59	3.66	3.28	3.51	3.16	3.16	3.16	- .35	-10.0
Erythrocyte	14.91	12.45	14.04	13.80	10.86	12.46	11.66	-2.14	-15.5
RG									
Plasma	3.12	2.98	3.66	3.25	2.97	3.11	3.04	- .21	- 0.65
Erythrocyte	14.46	15.62	14.04	14.71	15.57	15.94	15.76	+1.05	+ 7.1
PM (F)									
Plasma	3.59	3.48	3.57	3.54	1.76	1.29	1.53	-2.01	-56.8
Erythrocyte	11.76	13.04	11.18	11.99	9.55	8.84	9.20	-2.79	-23.3
JV									
Plasma	3.04	3.82	3.95	3.60	1.85	1.53	1.69	-1.91	-53.1
Erythrocyte	10.86	11.12	11.57	11.18	9.55	7.44	8.49	-2.69	-24.1
LM									
Plasma	4.89	4.43	4.49	4.60	-	3.69	3.69	- .91	-19.8
Erythrocyte	14.53	12.12	13.91	13.52	13.34	12.46	12.90	- .62	- 4.6
MM (F)									
Plasma	1.57	1.83	2.53	1.98	1.00	0.86	.93	-1.05	-53.0
Erythrocyte	16.40	15.62	12.61	14.88	12.95	-	12.95	-1.93	-13.0

Table VI (Con't.)

PARTICIPANTS' INITIALS	PRE-EXPOSURE SAMPLES			AVERAGE	POST-EXPOSURE SAMPLES		AVERAGE	DIFFERENCE	PERCENT DIFFERENCE
	1	2	3		1	2			
TR (F)									
Plasma	3.14	3.48	4.29	3.64	1.26	1.44	1.35	-2.29	-62.9
Erythrocyte	10.79	10.39	11.12	10.77	8.50	11.15	9.83	- .94	- 8.7
MR (F)									
Plasma	3.04	3.26	3.01	3.10	2.04	1.51	1.78	-1.32	-42.6
Erythrocyte	11.54	9.53	12.22	11.10	8.63	7.64	8.14	-2.96	-26.7
JP									
Plasma	4.09	4.59	3.95	4.21	3.29	3.50	3.40	- .81	-19.2
Erythrocyte	12.88	14.70	13.39	13.66	12.49	11.84	12.17	-1.49	-10.9
IG									
Plasma	3.99	5.14	5.06	4.73	-	3.88	3.88	.85	-18.0
Erythrocyte	10.49	9.47	12.35	10.77	9.94	11.46	10.70	.07	- 0.7
JR									
Plasma	4.10	2.49	2.17	2.92	2.89	2.16	2.53	- .39	-13.4
Erythrocyte	14.69	12.50	13.41	13.53	12.82	15.81	14.32	+ .79	+ 5.8
GG									
Plasma	2.60	1.94	1.51	2.02	1.94	.79	1.37	- .65	-32.2
Erythrocyte	11.44	9.81	12.43	11.23	9.42	9.45	9.44	- .79	- 7.0
MO									
Plasma	2.48	1.29	.72	1.50	1.40	.85	1.13	- .37	-24.6
Erythrocyte	10.53	7.12	7.00	8.22	5.62	6.03	5.82	-2.40	-29.2
CO									
Plasma	2.41	1.60	1.92	1.98	.99	.39	1.13	- .85	-42.9
Erythrocyte	9.23	7.12	8.37	8.24	6.54	6.03	6.29	-1.95	-23.7

Table VII

EXPOSURE OF RESIDUES OF GUTHION TO PICKERS

PARTICIPANTS' INITIALS	RESIDUES (in Mg.) FOUND AFTER 1 HOUR EXPOSURE					PERCENT DIFFERENCE	
	5.25 in.^{2} Each		Gloves (in Mg.)	Air		Plasma	RBC
	Arm	Head		Total	γ/Liter		
CO	0.027	0.022	38.1	0.027	0.15	-42.9	-23.7
CG	0.001	0.010	9.8	0.026	0.14	-32.2	- 7.0
MO	0.041	0.007	21.3	0.019	0.11	-24.6	-29.2
PM	0.004	0.002	32.8	0.024	0.13	-56.8	-23.3
JV	0.000	0.001	5.4	0.006	0.03	-53.1	-24.1
MM (F)	0.200	0.027	15.2	0.016	0.09	-53.0	-13.0
JR	0.001	0.016	36.9	0.064	0.35	-13.4	+ 5.8
MR (F)	0.004	0.015	22.6	0.034	0.19	-42.6	-26.7
EG	0.100	0.006	23.8	0.028	0.15	-18.0	- 0.7
JP	0.095	0.040	21.5	0.012	0.07	-19.2	-10.9

Table VIII

PERCENT OF SURFACE RESIDUES OF GUTHION REMOVED
WITH WATER AND BENZENE - BLOCK 1

DAYS INTERVAL	H_2O	LEAVES PhH	TOTAL PPM	H_2O	FRUIT PhH	TOTAL PPM
Control	39	61	0.16	-	-	0.01
0	49	51	106	40	60	0.14
1	30	70	37	18	82	0.67
3	58	42	99	40	60	0.42
7	71	29	59	31	69	0.35
9	88	12	176	26	74	0.34
11	75	25	66	16	84	0.63
15	77	23	50	31	69	0.69
23	87	13	51	-	-	-
30	72	28	32	29	71	0.40

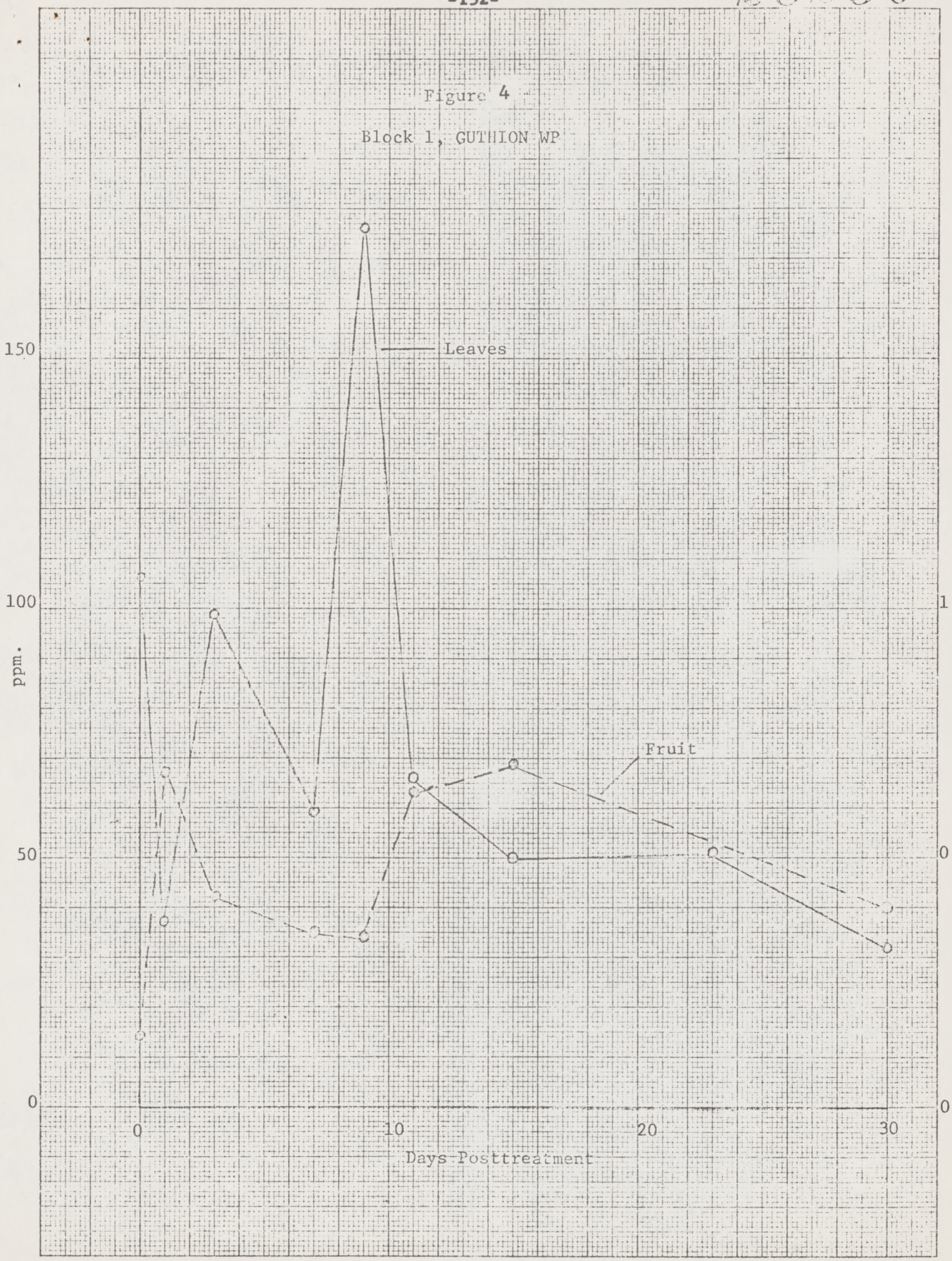
Table IX

COMPARISON OF GUTHION AND ETHION
SURFACE RESIDUES - AREA C, BLOCK 1

	PERCENT OF TOTAL SURFACE RESIDUE	
	Guthion	Ethion
Leaves:		
Water extraction	56	21
Benzene extraction	44	79
Fruit:		
Water extraction	20	23
Benzene extraction	80	77

Figure 4

Block 1, GUTHION WP



28250

Figure 5

Area A (Block I) GUTHION SC

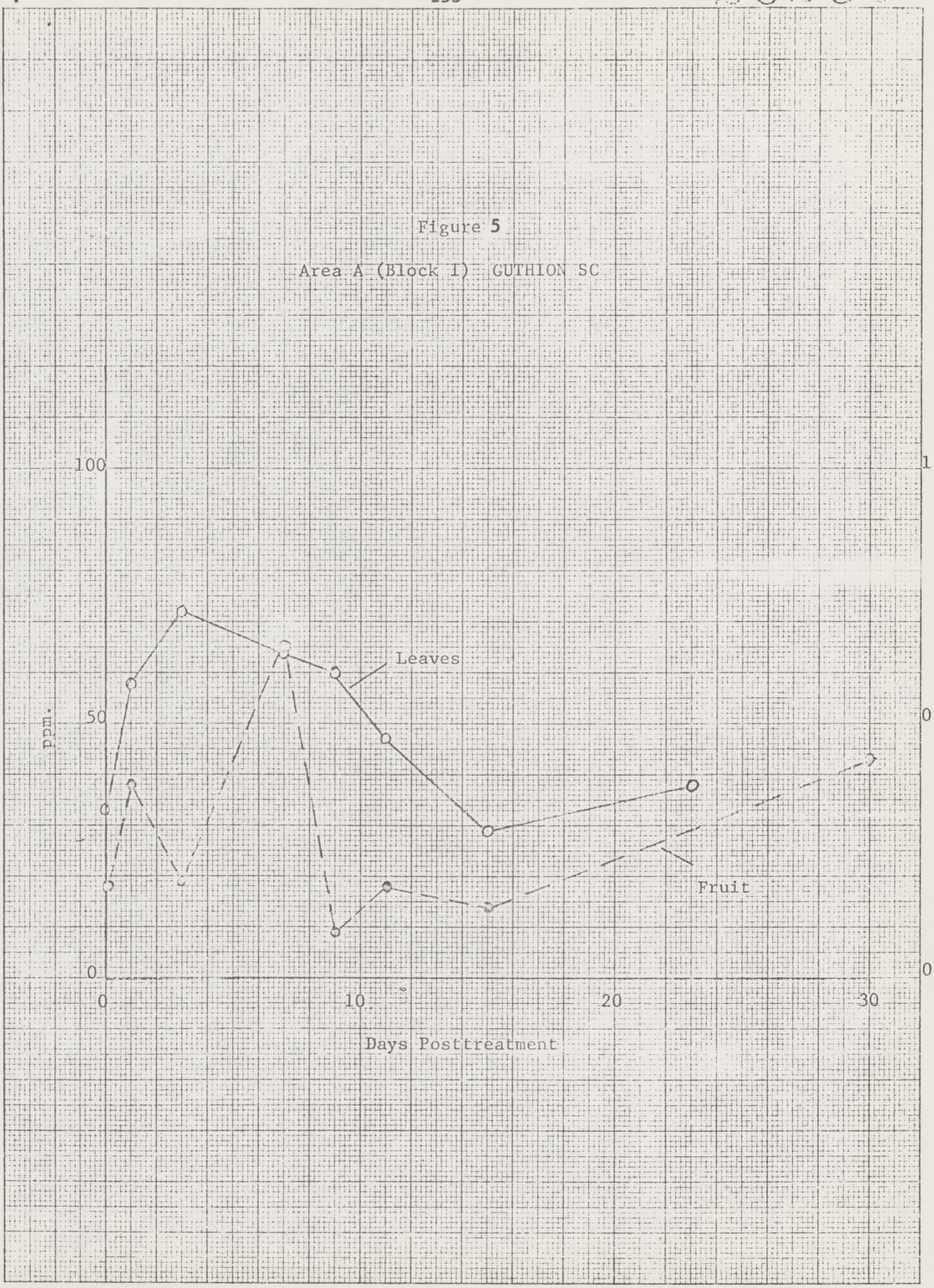


Figure 6

Area B (Block 1) Ethion WP

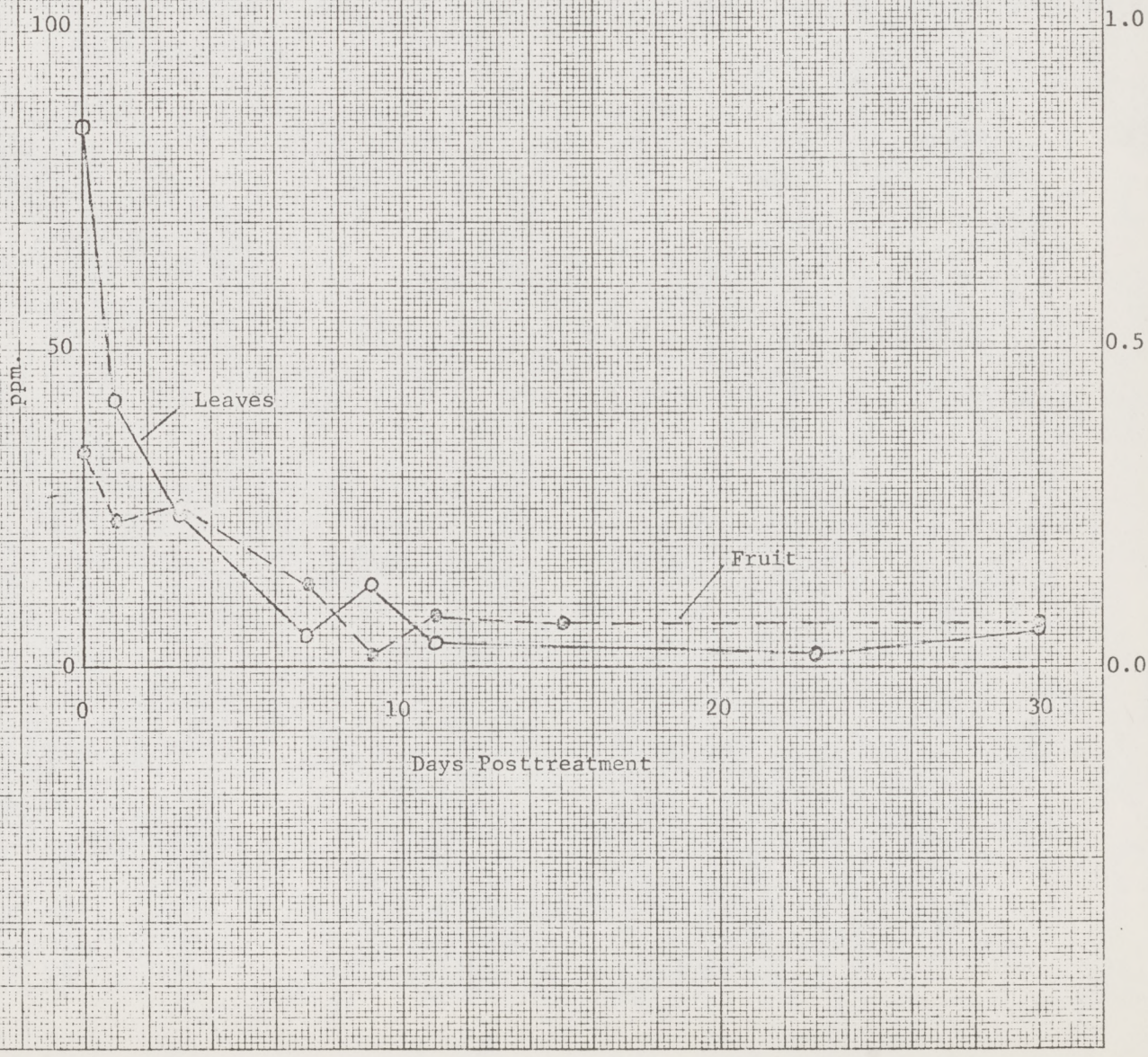


Figure 7

Area C (Block 1) GUTHION WP (Ethion)

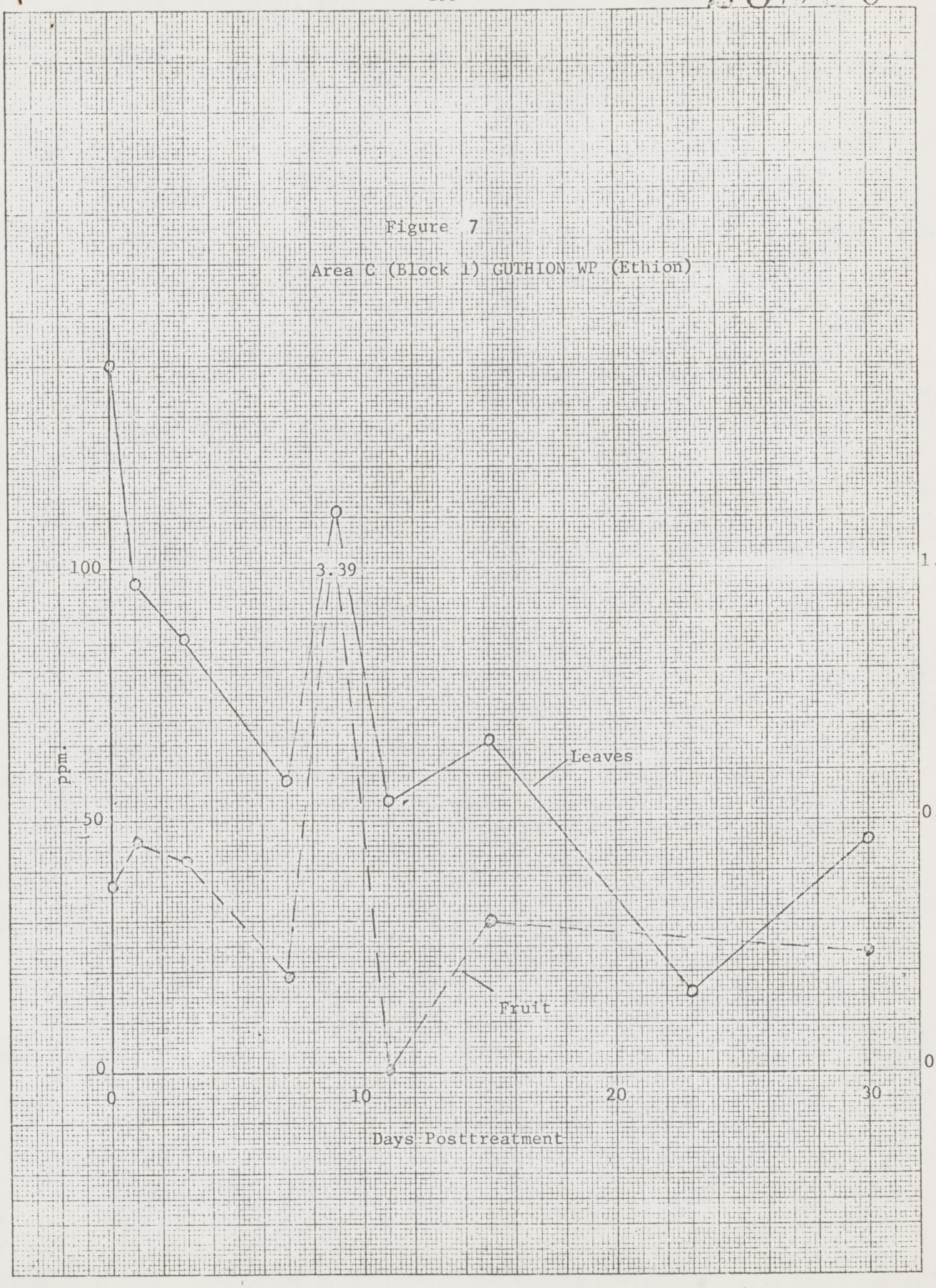


Figure 8

Area C (Block 1) - Ethion WP (GUTHION)

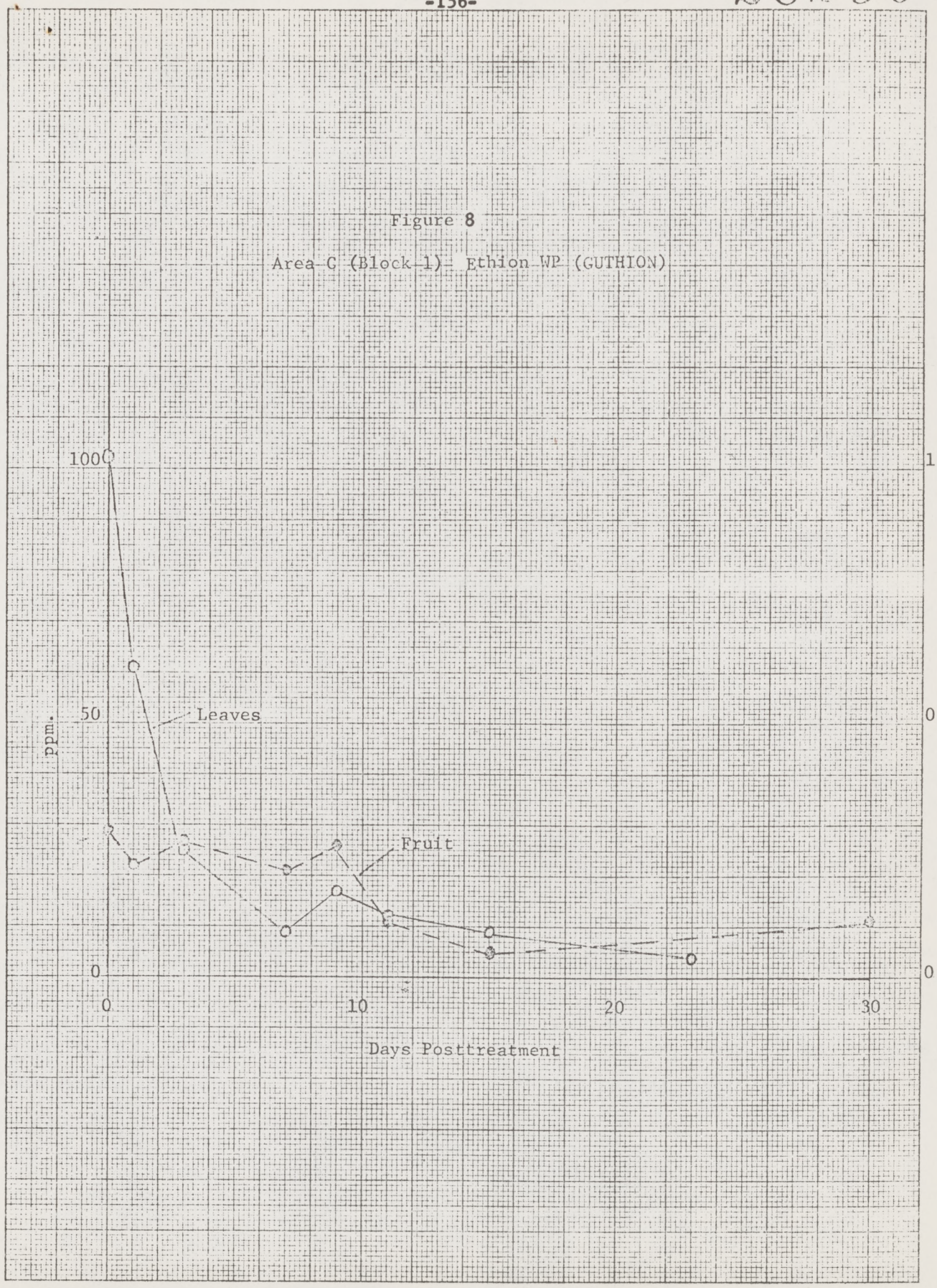


Figure 9

Block 2, GUTHION WP

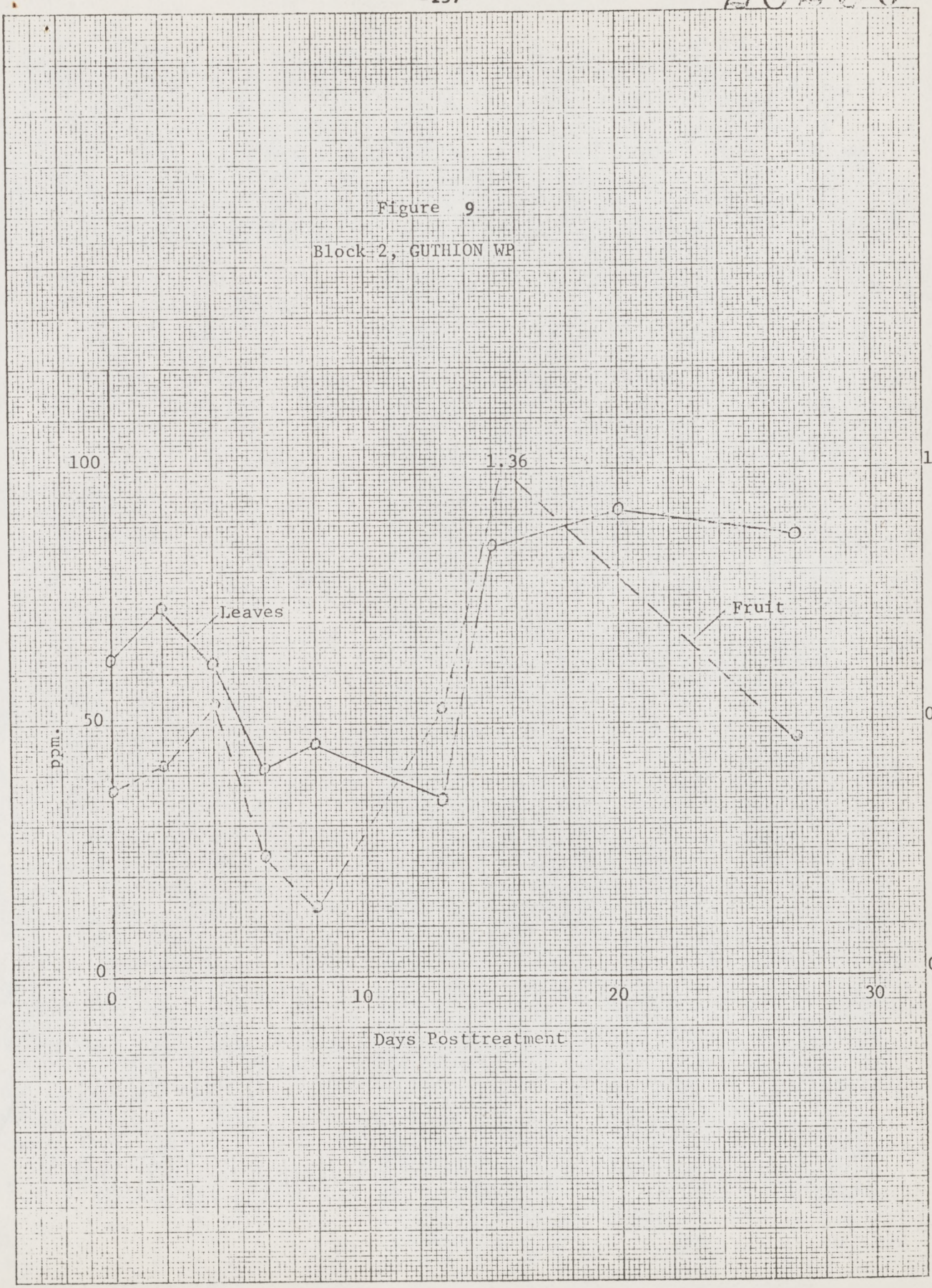


Figure 10

Area A (Block 2) GUTHION SC

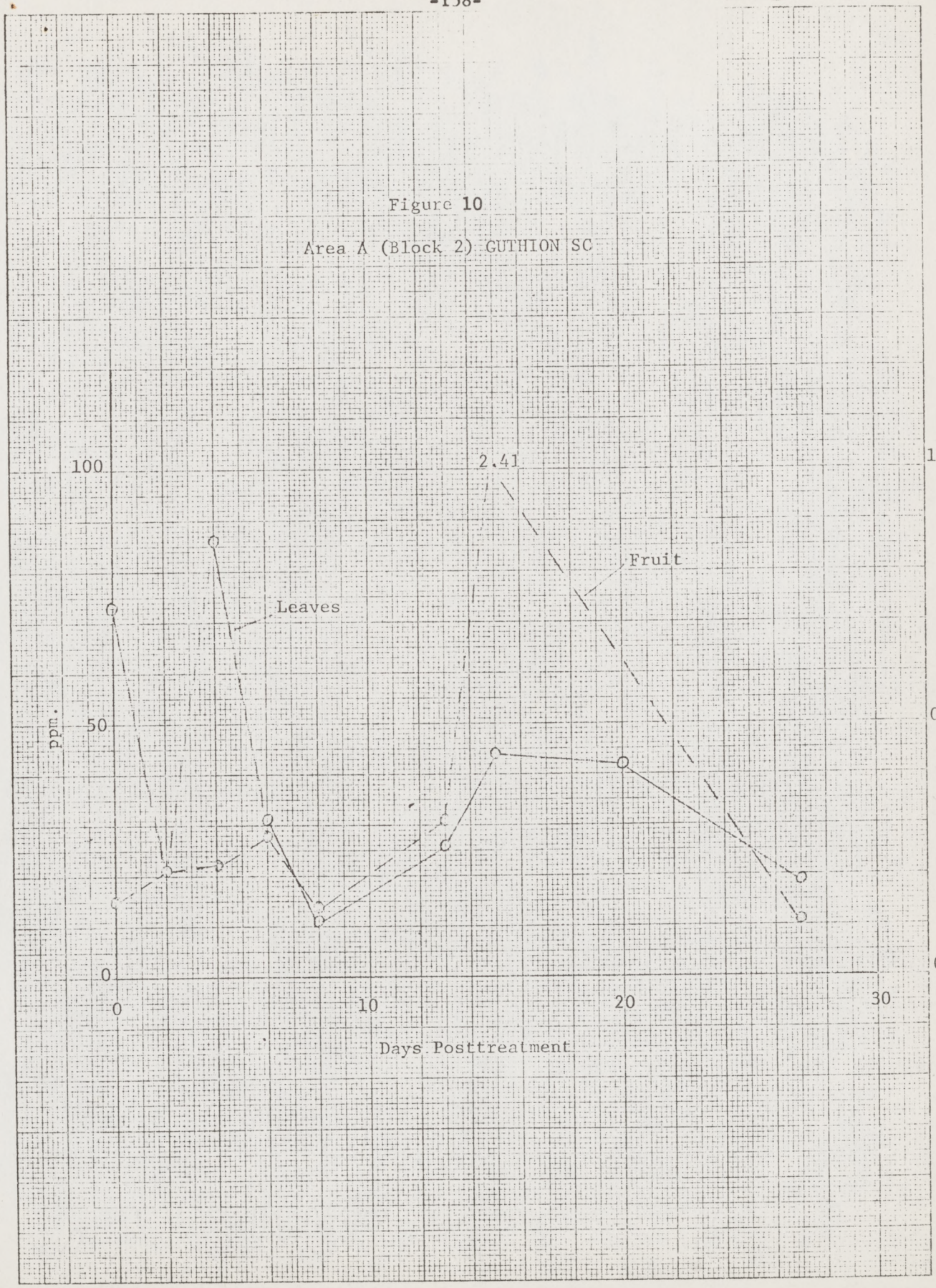


Figure 11

Area B (Block 2) Ethion WP

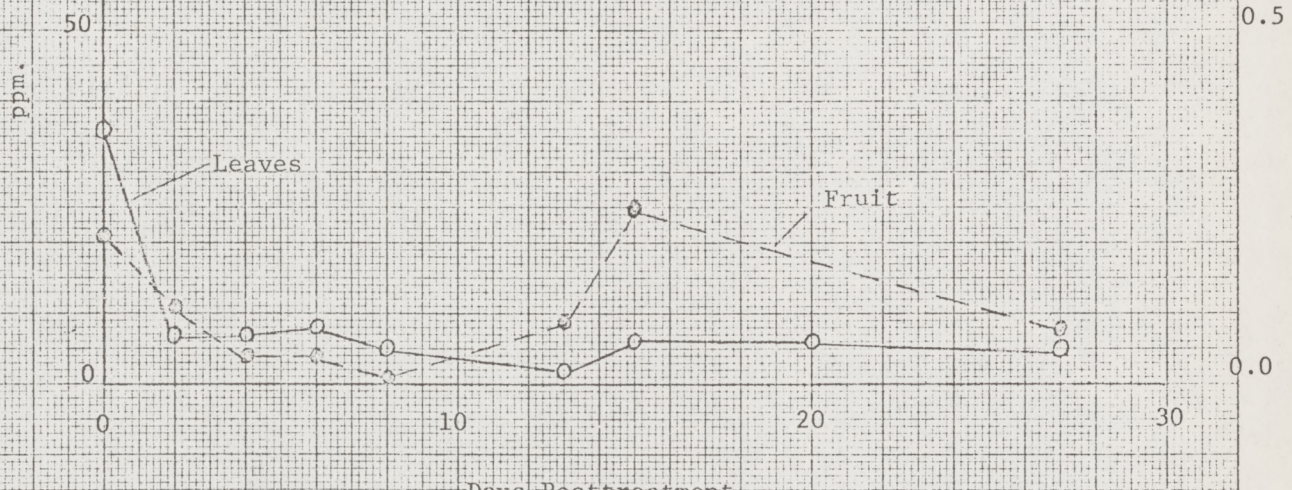


Figure 12

Area C (Block 2) GUTHION WP (Ethion)

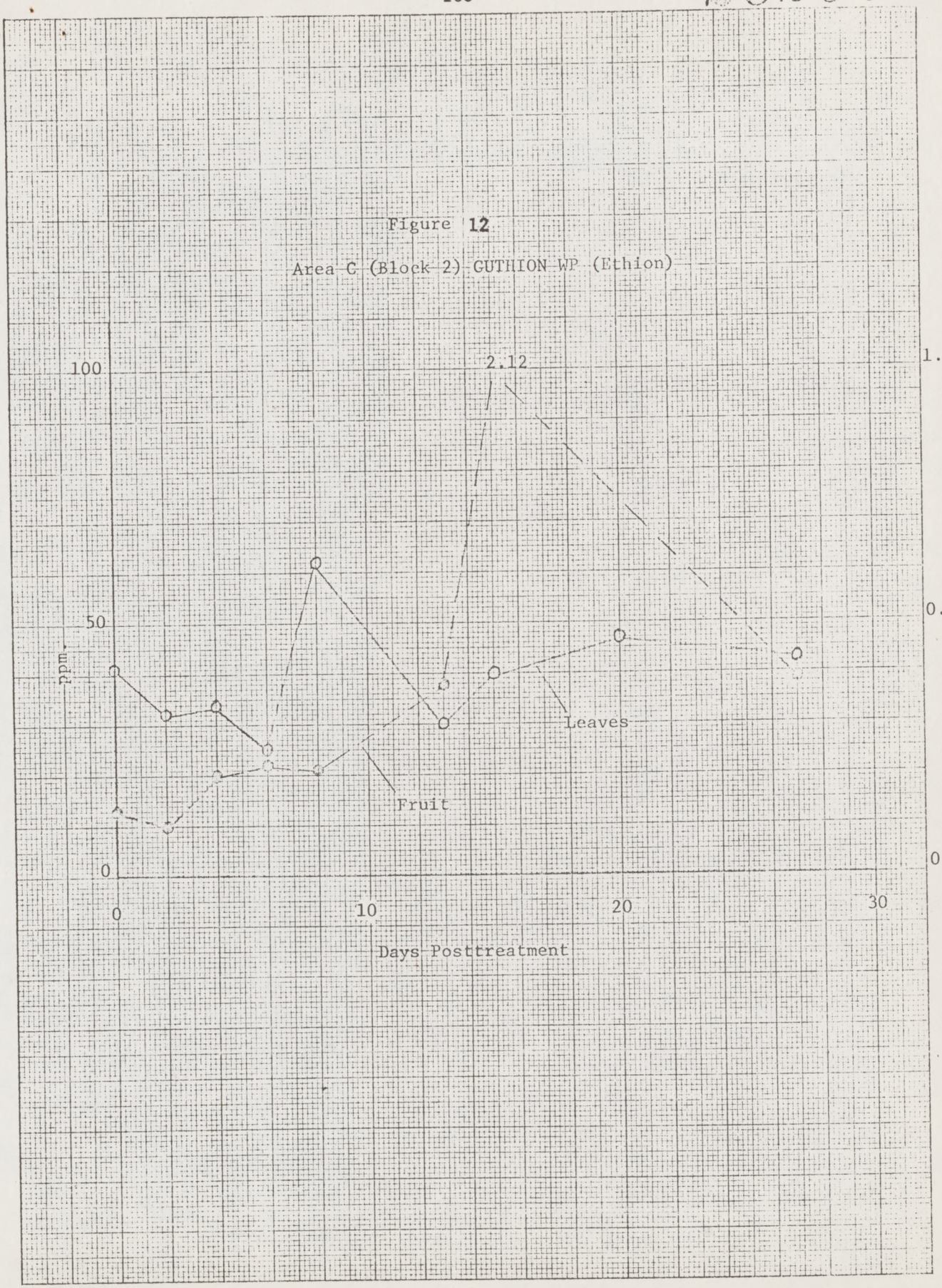
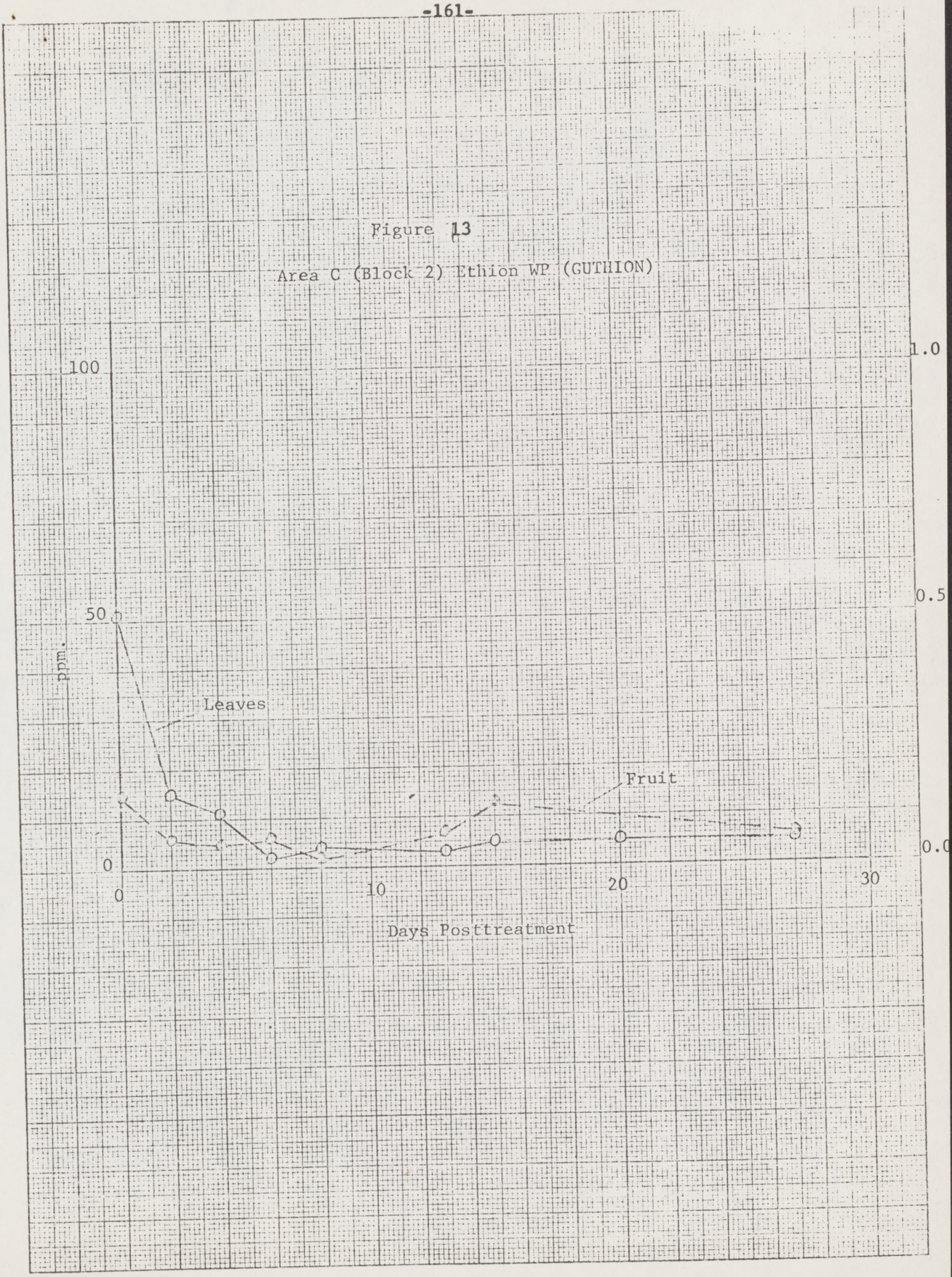


Figure 13

Area C (Block 2) Ethion WP (GUTHION)



Appendix 1

22250

MONTHLY CLIMATIC SUMMARY
FIELD STATIONS - DIVISION OF AGRICULTURAL SCIENCES
UNIVERSITY OF CALIFORNIA

Location: Lindcove
Observer: Phillip Tomlinson
Instrumentation data on back of sheet

Date Tabulated: August
Date Received: Kilburn
Period of Report: August 1[illegible]

*Date	n	T max	T min	Tsp max	Tsp min	RH+	RH-	ST max	ST min	E max	E min	Wind	WV_n	V_d	V_n
1		93	58	94	58	27%	94%	94	86	113	56	5.6	1.1	NE	S
2		95	60	97	62	38%	82%	94	86	122	57	4.4	.9	NW	N
3		99	74	100	76	27%	81%	95	86	120	-	-	-	-	.
4		96	64	97	66	34%	90%	95	88	118	65	5.4	1.5	SW	S
5		93	62	94	62	35%	76%	94	87	116	60	4.2	1.8	S	SW
6		94	61	95	63	36%	95%	94	87	114	58	5.4	.9	SW	-
7		97	63	98	65	37%	95%	95	87	117	61	4.6	.9	S	SE
8		98	63	100	64	33%	93%	96	88	118	60	4.6	.9	SW	W
9		101	62	103	70	28%	85%	97	88	122	60	3.3	.9	SW	W
10		114	64	105	68	54%	78%	97	88	122	63	-	-	-	.
11		102	72	104	72	30%	-	97	90	122	74	5.4	.3	SW	N
12		102	68	104	70	26%	90%	98	90	121	65	5.1	.6	S	N
13		100	67	102	69	32%	84%	98	90	120	64	4.2	.4	SW	S
14		100	64	101	65	26%	84%	98	90	118	62	4.2	.4	SE	S
15		100	64	101	68	30%	83%	97	90	120	62	4.5	.9	SW	SE
16		100	69	101	71	30%	75%	98	90	119	58	4.7	1.2	SW	W
17		99	60	100	61	38%	99%	98	88	119	58	3.6	1.1	NW	N
18		98	70	99	70	33%	91%	98	91	117	68	5.7	.9	N	NW
19		97	69	98	69	35%	85%	97	90	116	66	5.2	1.3	N	SE
20		95	66	96	65	32%	74%	96	89	116	63	5.6	.9	NW	N
21		95	62	96	64	27%	89%	96	88	114	60	4.6	.7	SW	S
22		95	63	96	63	34%	90%	96	88	114	60	4.5	.9	SW	N
23		93	61	95	62	35%	95%	95	87	119	58	4.5	.6	NW	N
24		95	-	96	60	-	-	94	87	115	-	4.0	.3	S	S
25		93	67	97	66	-	-	94	88	119	63	4.1	.8	N	N
26		91	60	98	60	-	-	93	85	110	63	4.2	1.0	NW	NW
27		93	86	94	66	-	-	96	88	115	64	3.6	.6	SW	N
28		93	64	96	65	-	-	94	88	117	62	4.8	.7	S	NW
29		93	60	96	64	-	-	94	88	114	63	4.5	1.3	NW	SE
30		89	60	90	60	-	-	93	85	113	57	4.5	.6	SW	NE
31															
Sum		2909	1863	2937	1965	757	1912	2869	2643	3414	1723	129.0	24.		
Mean		97	62	98	66	33	87	96	88	114	62	4.6	.9		

* See back of sheet for legend.

Appendix 1 (con't.)

28250

MONTHLY CLIMATIC SUMMARY
FIELD STATIONS – DIVISION OF AGRICULTURAL SCIENCES
UNIVERSITY OF CALIFORNIA

Location: Lindcove
Observer: Phil Tomlinson
Instrumentation data on back of sheet

Date Tabulated: September 1970
Data Recorder: Kilburg
Period of Report: September 1970

*Date	R	T max	T min	Tsp max	Tsp min	RH+	RH–	ST max	ST min	K max	K min	WVd	WVn	Wd	Wn
1		92	58	92	59	34%	100%	92	85	112	58	5.1	.3	NW	
2		31	58	92	58	28%	89%	91	84	112	54	4.6	.6	NW	
3		92	56	93	58	26%	88%	90	83	111	54	4.5	.8	NW	
4		87	56	86	58	36%	88%	90	83	117	54	4.8	2.9	NW	
5		78	53	78	52	40%	87%	87	82	99	49	6.3	.9		
6		89	55	90	58	27%	75%	88	80	110	52	3.0	2.7	SW	
7		-	-	-	-	-	-	-	-	-	-	-	-	-	-
8		96	61	97	64	34%	-	90	82	114	60	4.2	.5	SW	S
9		97	63	99	66	35%	83%	91	84	118	63	4.8	.7	SW	
10		100	65	101	68	29%	78%	92	85	121	63	3.5	.4	NW	
11		98	64	100	67	31%	80%	92	85	118	62	4.5	.7	NW	
12		95	60	96	64	13%	83%	91	85	112	58	5.2	2.3	SW	
13		80	54	79	55	35%	38%	88	83	103	50	3.9	.4	SW	
14															
15															
16															
17															
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22															
23															
24															
25															
26															
27															
28															
29															
30															
31															
SUM															
MEAN															

* See back of sheet for legend.

Appendix 3

INSTRUMENTATION DATA

Temperature Recorder:

Type _____ Serial No. _____

Date of last calibration _____

Remarks:

Wind and Special Temperature Recorder:

Type _____ Serial No. _____

Date of last calibration _____

Remarks: _____

Anemometer Serial No. _____

Date of last Calibration _____

Remarks:

Rain Gage:

Type _____ Serial No. _____

Date of last calibration _____

Remarks:

LEGEND

R – Rainfall in inches, 24 hour period.
T max – Maximum temperature °F at 60 inches during 24 hour period (Midnight to Midnight)
T min – Minimum temperature °F at 60 inches during 24 hour period (Midnight to Midnight)
SP Tmax – Special height maximum temperature °F during 24 hour period (Midnight to Midnight)
SP Tmin – Special height minimum temperature °F during 24 hour period (Midnight to Midnight)
RH+ – Relative humidity at maximum dry bulb
RH- – Relative humidity at minimum dry bulb
ST max – Maximum soil temperature °F at 6 inches during 24 hour period (Midnight to Midnight)
ST min – Minimum soil temperature °F at 6 inches during 24 hour period (Midnight to Midnight)
B max – Maximum black globe temperature °F during 24 hour period (Midnight to Midnight)
B min – Minimum black globe temperature °F during 24 hour period (Midnight to Midnight)
WV_d – Average wind velocity MPH 6 a.m. to 6 p.m.
WV_n – Average wind velocity MPH 6 p.m. to 6 a.m.
D_d – Prevailing wind 6 a.m. to 6 p.m.
D_n – Prevailing wind 6 p.m. to 6 a.m.

DETERMINATION OF THE HAZARDS TO WORKERS PICKING CITRUS TREATED WITH GUTHION SPRAY CONCENTRATE FORMULATION

(Report of Chemagro Corporation)

I. Introduction

Treatment of citrus groves with GUTHION has been a practice in California for many years. The pre-harvest interval on the Federal Label is 7 days. This interval has not resulted in any toxicity problems for workers in the past. However, in May, 1970, the California Department of Public Health investigated an incident in Tulare County, California where 16 orange pickers became ill.

It was known that the citrus grove where the workers were picking had previously been treated with parathion, ethion and GUTHION. It was also known that the workers had been previously exposed to delnave, dimethoate and probably other compounds.

As a result of the incident in Tulare County, the California Department of Agriculture and the California Department of Public Health increased the 7-day pre-harvest interval to 30 days. In view of the fact that the workers involved in the Tulare County incident had been exposed to a number of pesticides we decided to carry out further tests to see if a 30-day interval for GUTHION was really necessary. The test protocol that was developed provided for cholinesterase tests for workers and collection of residue data for fruit, foliage, gloves, skin patches, urine and air. They also requested residue data to show what effect other pesticides had on residues of GUTHION and vice versa. Since GUTHION and ethion are most frequently used in combination on citrus, ethion was chosen as the other pesticide used in our study.

Briefly, the test consisted of treating a block of orange trees with GUTHION and several smaller areas with GUTHION and ethion to get a side-by-side comparison of residues. The Wettable Powder (WP) formulation of GUTHION was used in a prior study, Chemagro Report No. 28250 - October 7, 1970. The Spray Concentrate formulation was used in this study. Although the maximum rate allowed on the Federal Label is 6 ounces per 100 gallons and 2,000 gallons per acre, the use rate is generally 4 ounces active per 100 gallons and 800 to 1,000 gallons per acre. In this test a rate of 4 ounces per 100 gallons and 900 gallons per acre was used (2.25 #/acre).

Three main blocks of trees were included in this study. Block 1 and four smaller areas of trees were treated on the same day to obtain side-by-side comparisons of residues of GUTHION SC in Block 1 and the effect of ethion EC on residues of GUTHION SC in the smaller areas.

It was originally planned for the workers to enter Block 1 on the seventh day, but the citrus packer backed out of the test and would not accept oranges from Block 1. Therefore, Block 2 was treated with GUTHION SC in the same way as Block 1. Block 3 was then treated on the tenth day after Block 2. Smaller areas were not treated simultaneously with Blocks 2 and 3, but only with Block 1.

Workers entered Block 2 on the seventh day after treatment and picked on days 7 and 10 through 14. Workers then entered Block 3 on the seventh day after treatment and picked on days 7 through 11. Plasma and erythrocyte cholinesterase baselines were established for each worker prior to his entering Block 2 and twice after entering Block 3. The assumption was that if there was going to be an effect, it would appear in the first 10 days.

Throughout the test, residue samples of leaves and fruit were collected from Block 1 and the corresponding smaller areas and were then related to residues from Blocks 2 and 3. Surface residues on leaves and fruit were determined by washing with water followed by benzene. Residues removed by water may be potentially hazardous to workers, since the residues were likely to be easily removed. Samples were also collected to measure exposure through the skin and by inhalation.

The details of the procedures are now described.

II. Experimental

Before the study was started, a protocol was written and sent to the State of California for their approval and suggestions. This protocol has been followed as closely as possible throughout the study.

Orange Grove

A grove of oranges which had not been sprayed with cholinesterase-inhibiting pesticides for at least 30 days was located in the Whittemore Orchard, Visalia, California. The trees showed an average cover of dust. Block 1 was sprayed with GUTHION SC, 4 ounces active per 100 gallons, 900 gallons per acre on September 14, 1970. Block 2 was sprayed on September 18, 1970. Block 3 was sprayed on September 28, 1970. Treatment for all blocks were identical in every way.

Four additional areas of 8 trees were treated with Block 1 with the following formulations:

AREA	FORMULATION OUNCES ACTIVE/100 GAL.	VOLUME GAL./ACRE
A	GUTHION SC - 4 Ethion EC - 4	900
B	Ethion EC - 4	900
C	GUTHION SC - 4	1800
D	GUTHION SC - 4 Ethion WP - 4	900

Pre-treatment samples of leaves and fruit were collected from Block 1 and the corresponding small ares. Post-treatment samples were collected from Block 1 and the corresponding areas at 0, 6 and 15 days; Block 2 at 0, and 3 and 7 days; and Block 3 at 1 and 6 days at the time of writing this report. Samples were collected to 30 days.

Four average size leaves were collected from each quadrant of each of 24 trees in the main blocks and from each of the 8 trees in the smaller areas. The leaves were collected so that the entire sample was comprised of a representative number from the interior and exterior portion of the trees. Triplicate samples were collected at all intervals.

Five pounds of fruit were collected at the same time in the same manner as the leaves. Care was taken to avoid loss of surface residue in the form of dust. Therefore, the fruit and leaves were each put into plastic bags and then put into a cloth residue bag.

Samples of leaves and fruit were also collected from the corresponding areas, A,B, C and D. Residue samples were taken from Area C to show if residues were higher due to the 1,800 gallon per acre volume as compared to 900 gallon per acre.

Workers

Workers of age 21 years of age or older who had no recent exposure to cholinesterase-inhibiting pesticides were chosen. Baseline cholinesterase levels were established for 15 workers. It was orginally planned to collect pre-treatment blood samples from each worker 7, 5 and 3 days prior to entering the treated field. The first pre-treatment sample was collected 9 days before the test, but before a second sample was collected, some workers dropped from the test on their own initiative, and other workers were added to ensure at least 15 workers at the start of the test. A second sample was collected 4 days before the test, a third 2 days before the test and a fourth sample on the day of the test. During the pre-treatment time, workers continued to drop from the test and new ones were added. Consequently, on the first day of picking the original plan of 3 pre-treatment intervals of 7, 5 and 3 days was altered. Workers whose cholinesterase levels were not within the normal range would have been eliminated from the test. During the time the baseline values were being established, the workers were not allowed to work in orchards, groves etc. which had been sprayed with cholinesterase-inhibiting pesticides.

Starting the first day of picking, an attempt to collect a 24-hour urine sample from each worker was made. Workers were supplied with urine collection bottles. An aliquot was taken and shipped to Kansas City for analysis.

The blood samples were taken at the end of a full day of labor on the third and sixth day after picking in Block 2 and on the second and fifth day after picking in Block 3.

Blood samples were collected by venipuncture. The analytical method was the pH stat assay for human blood cholinesterase recommended by the United States Public Health Service. The analytical values reported are the

pH stat unit values (μ mol acetylcholine/min/ml). An average pre-exposure value was calculated for each individual. The post-exposure values were compared to the average pre-exposure value to measure effect.

On the first day of picking in Block 2, all workers were given new cotton gloves. The gloves were collected at the end of the first day of picking. Thereafter, the pickers wore their own gloves, and in some cases, they did not wear any gloves. However, each day two workers were chosen to wear new cotton gloves, skin patches, one on the forearm and one on the head, and to carry an air sampling device, Telmatic 150 Air Sampler. The gloves, patches and air sampler were worn 60 minutes on each day. The gloves, patches and mineral oil from the air sampler trap were sent to Kansas City for analysis. On each successive day the time which the workers wore the gloves, patches and air sampler was an hour later in the day to observe any differences in residues as the day progressed.

The air flow in the sir sampling device was 2.8 liters per minute, and was collected near the worker's face. Each gauze patch was 5.25 square inches.

All the orange pickers spent from seven to nine hours in the grove each day.

Weater Data

The weater data were recorded in the Monthly Climatic Summary, Lindcove, California, a field station of the University of California. There was no rainfall during the test at the time of writing this report. The official record of the field station will be collected at the end of the test.

Analysis of Residue Samples - Procedure

Leaves:

1) The leaves and bag were weighed.

2) The bag was cut into 4 pieces and rinsed throughly in 400 ml. water, followed by a second rinse in a second 400 ml. water.

3) The leaves were rinsed in the same manner in bunches of 20 - 30 leaves, using the same water that was used for the bag rinses.

4) Contact time in all water rinses was 10 - 20 seconds.

5) The two beakers of water from the bag and leaf rinses were combined and adjusted to 800 ml. A 200-ml. aliquot was extracted with 200 ml. chloroform.

6) A 100-ml. aliquot of the chloroform extract from the leaves and bag was evaporated to dryness and the residue was re-dissolved in 2 ml. acetone.

7) The leaves were then washed in two 400 ml. portions of benzene.

8) The benzene extracts were combined and diluted to 800 ml. A 100 ml. aliquot was evaporated to dryness and the residue was dissolved in 2 ml. acetone.

Oranges:

1) The oranges and bag were weighed.

2) The bag was treated in the same manner as above.

3) Each orange was stabbed with a micro spatula with a sharp point and swirled ten times in 400 ml. of water, and then in a second 400 ml. of water. Contact time in each water wash was 5 - 10 seconds.

4) Step 3 was repeated for all oranges in the sample.

5) The two water extracts were combined and diluted to 800 ml.

6) A 200 ml. aliquot was extracted with 200 ml. chloroform. A 100-ml.aliquot chloroform was evaporated to dryness and the residue was dissolved in 2 ml. acetone.

7) The oranges were then rinsed in two 400 ml. portions of benzene in the same manner as in Step 3.

8) The benzene extracts were combined and diluted to 800 ml. A 100 ml. aliquot was evaporated to dryness and the residue was re-dissolved in 2 ml. acetone.

Gloves:

1) Each pair was immersed and mixed in 400 ml. chloroform for 3 - 5 minutes.

2) The chloroform was squeezed from the gloves.

3) A 100 ml. aliquot was evaporated to dryness and the residue was re-dissolved in 2 ml. acetone.

Mineral Oil:

1) The contents (30 ml.) of the trap from the air sampling unit were emptied and the trap was rinsed twice with 5 - 10 ml. mineral oil. The rinses were combined with the original contents and shipped in a stoppered glass jar to Kansas City.

2) The contents of the jar were rinsed into a separatory funnel, using 200 ml. Skellysolve B.

3) The Skellysolve B was extracted with 100 ml. acetonitrile.

4) A 50-ml. aliquot of acetonitrile was evaporated to dryness and the residue was re-dissolved in 2 ml. acetone.

5) The Skellysolve B extract was placed in a tared, round-bottomed flask.

6) All the Skellysolve B was evaporated, and the weight of the mineral oil was determined by difference in flask weight.

Patches:

1) The patch (gauze) was placed in a 4-ounce bottle with 50 ml. chloroform.

2) The bottle was stoppered and shaken for five minutes.

3) A 25 ml. aliquot was evaporated to dryness and the residue was re-dissolved in 2 ml. acetone.

Gas Chromatographic Procedure

Column:	14 inches by 2 mm. i.d. glass, packed with 5% D.C. 200 on 70 - 80 mesh Chromosorb G
Temperature:	Oven 220°C Inlet 270°C Detector - 290°C
Carrier Gas:	Helium, 60 ml./minute. 40 psig.
Instrument:	Hewlett-Packard Model 5750, dual flame instrument modified for thermionic flame operation.

Four microliters of each acetone solution were injected and compared to a composite standard of GUTHION (6.25 microgram/ml.) and ethion (2.5 microgram/ml.). Acetone solutions containing high residues were diluted and compared to injected standards.

Calculations

Parts per million (ppm.) for both GUTHION and ethion were calculated from the general equation:

$$ppm = \frac{\text{Sample Area}}{\text{Standard Area}} \times \frac{\text{Nanograms Standard Inj.}}{\text{Wt. of Sample (g.)}} \times \frac{\text{Final Volume (ml.)}}{\text{Microliters Inj.}} \times \text{Aliquot Fraction}$$

III. Results and Discussion

The pre-exposure blood samples were taken at the indicated times shown in Table . The intervals for both Arebalo's are prior to their entering Block 3.

The results of all cholinesterase determinations are summarized in Table . The zero day pre-exposure interval is not shown for the following

reason. During that particular week, the Federal Public Health Service submitted referee samples to the Tulare Clinical Laboratories for acetylcholinesterase determination. The laboratory director, Dr. David B. Cooke, stated that the values reported for that day appeared to be in error. The values for erythrocytes appeared to be lower than expected, while the plasma values appeared to be normal. At the writing of this report, the values for that day are still questionable and, therefore, are omitted for calculation of the pre-exposure average value for each individual. The pre-exposure averages were plasma, 3.23 and erythrocytes, 12.57 for males and 2.52 and 12.60 for females.

Two post-exposure values from Block 2 and the first post-exposure value from Block 3 were used to calculate the post-exposure averages. The post-exposure averages were plasma, 3.13 and erythrocytes 10.22, for males and 3.00 and 11.02 for females. The plasma and erythrocyte post-exposure values for males were 96.9% and 81.3% of the pre-exposure averages. The plasma and erythrocyte post-exposure values for females were 119% and 87.5% of the pre-exposure avarages.

The fourth post-exposure value was not completed at the writing of this report. An evaluation of the post-exposure values for each worker cannot be made until all data have been collected. (By the time the CSP report was written, these additional values have been obtained and were added to the table).

A summary of the exposure to workers is shown in Tables XII AND XIII. The residues in the gloves after a full day's picking are shown in Table XII. The percent difference for cholinesterase levels is calculated from the averages of the pre and post-exposure values for each worker. A relationship between higher residues in the gloves and greater depression of cholinesterase was not observed.

In Table XIII, the data from gloves, patches and inhalation for two workers each day for a one-hour period are compared to the worker's changes in average cholinesterase levels. The correlation between exposure and cholinesterase depression is unclear and may be due to the fact the exposures were measured for a short period of time and may not be a representative sample.

Urine samples have not yet been analyzed. A fluorometric method is currently being developed.

Residues of GUTHION were more likely to wash off leaves than fruit. About 75% of the residue of GUTHION on leaves was removed with water, whereas, only about 30% was removed from fruit with water. For ethion residues, about 20% is removed from leaves with water, and less than 5% is removed from fruit with water.

The residues of GUTHION in Blocks 1, 2 and 3 and the corresponding areas where ethion was used in combination are shown in Figures #14 through #22 . Enough data are not available for a final evaluation, but residues of GUTHION in Blocks 1, 2 and 3 show a steady decline. Residues of ethion

also show a steady decline. It is not yet clear what effect residues of ethion have on residues of GUTHION and what differences in residues occur when GUTHION is applied using twice the volume of spray mixture.

IV. Summary

Orange trees were treated with GUTHION SC at the rate of 4 ounces active per 100 gallons and 900 gallons per acre. Smaller areas of trees were treated at the same rate with (1) GUTHION SC and ethion EC, (2) ethion EC, (3) GUTHION SC but with 1,800 gallons per acre, and (4) GUTHION SC and ethion WP. Surface residues on leaves and fruit were determined by first washing with water and then with benzene. Residues removed with water were likely to be easily removed, and thereby, a potential hazard to workers. The remainder of the residue was removed with benzene.

Baseline cholinesterase values from plasma and erythrocytes were established for 15 workers. The workers entered two main blocks of trees treated with GUTHION SC 4 ounces per 100 gallons and 900 gallons per acre. Cholinesterase levels were determined twice in Block 1 and twice in Block 2.

Residues from GUTHION SC showed a gradual decline through 15 days. All the data were not collected and a complete study of the residues is not yet available.

The average post-exposure cholinesterase levels of plasma and erythrocytes after eight days of picking were 96.9% and 81.3% of the pre-exposure average for males. For females, the post-exposure levels were 119% and 87.5% of the pre-exposure averages. The fourth post-exposure value for cholinesterase was not yet available and a final evaluation was not possible at the writing of this report.

The correlation between exposure and cholinesterase depression is unclear and may be due to the fact the exposures were measured for a short period of time and may not be a representative sample.

The residues in the air were usually less than 0.1 micrograms per liter. Only two samples out of ten showed residues of 0.1 micrograms per liter.

###

Table X

PRE-EXPOSURE COLLECTION DATES OF BLOOD FROM WORKERS

PARTICIPANT'S INITIAL	INTERVALS (IN DAYS) BEFORE ENTERING BLOCK 1
MC	- 4,2,0
NH	- 4,2,0
JdL	9,4,2,0
JM	9,4,-,0
RP	9,4,2,0
RR	9,4,-,0
LR	9,4,2,0
VS	9,4,-,0
JE	0,4,2,0
AT[1]	- - 2,0
ET[1]	- - 2,0
EA	- 4,2,0
EA	- 4,2,0
EA[2]	4,1
AA[2]	4,1

[1] Added after other workers dropped from test.

[2] Added after other workers dropped from the test, and the intervals listed are prior to entering Block 3 (they did not pick in Block 2).

Table XI

PLASMA AND ERYTHROCYTE CHOLINESTERASE VALUES FROM WORKERS IN GUTHION SC - CITRUS STUDY

PARTICIPANT'S INITIAL	PRE-EXPOSURE SAMPLES			AVE-RAGE	POST-EXPOSURE SAMPLES				AVE-RAGE	DIFFER-ENCE	PERCENT DIFFER-ENCE
	1	2	3		1	2	3	4*			
MC											
Plasma	2.21	1.00	-	2.21	2.22	1.85	2.18	1.53	2.08	-0.13	- 5.9
Erythrocyte	10.30	11.92	-	11.11	7.54	3.85	3.53	2.68	4.97	-6.14	-55.3
NH											
Plasma	2.99	2.50	-	2.69	2.94	2.40	5.44	2.57	3.59	+0.90	+33.5
Erythrocyte	14.08	11.50	-	12.79	11.57	7.42	13.18	4.99	10.72	-2.07	-16.2
JdE											
Plasma	3.26	3.86	2.33	3.57	3.17	3.44	3.41	3.06	3.34	-0.23	- 6.4
Erythrocyte	9.60	12.16	10.21	10.65	10.72	10.43	8.84	6.14	10.00	-0.65	- 6.1
JM											
Plasma	1.62	2.73	-	2.17	4.21	2.94	2.35	2.25	3.17	+1.00	+46.1
Erythrocyte	12.61	8.95	-	10.78	13.78	9.43	8.56	5.88	10.59	-0.19	- 1.8
RP											
Plasma	4.27	3.13	3.65	3.70	2.72	2.46	2.54	2.04	2.57	-1.13	-30.5
Erythrocyte	11.07	12.41	8.91	10.79	10.92	7.36	6.59	10.88	8.29	-2.50	-23.2
RR											
Plasma	4.78	5.01	-	4.89	4.91	5.10	5.03	4.64	5.01	+0.12	+ 2.5
Erythrocyte	13.34	17.65	-	15.33	13.65	14.33	10.75	10.36	12.91	-2.42	-15.8
LR											
Plasma	1.11	1.68	3.80	1.96	1.61	1.36	1.52	1.23	1.50	-0.46	-23.5
Erythrocyte	11.07	12.03	11.57	11.55	11.37	7.42	(A)	6.01	9.40	-2.15	-18.6

Table XI (Con't.)

PARTICIPANT'S INITIAL	PRE-EXPOSURE SAMPLES			AVE-RAGE	POST-EXPOSURE SAMPLES				AVE-RAGE	DIFFER-ENCE	PERCENT DIFFER-ENCE
	1	2	3		1	2	3	4*			
VS (F)											
Plasma	1.86	3.42	-	2.64	5.39	2.26	1.83	2.13	3.16	+0.52	+19.7
Erythrocyte	10.94	13.79	-	12.44	12.31	8.83	8.70	5.88	9.95	-2.49	-20.0
JE											
Plasma	3.91	3.50	-	3.70	2.72	(B)	2.77	2.72	2.75	-0.95	-25.7
Erythrocyte	16.56	14.70	-	15.83	13.91	(B)	10.88	7.80	12.40	-3.43	-21.7
AT											
Plasma	4.80	-	-	4.80	3.42	3.72	3.88	-	3.67	-1.13	-23.5
Erythrocyte	14.20	-	-	14.30	13.32	7.35	6.85	5.37	9.17	-5.13	-35.9
ET (F)											
Plasma	1.60	-	-	1.60	3.67	2.77	2.92	-	3.12	+1.52	+95.0
Erythrocyte	11.51	-	-	11.51	11.44	8.28	8.02	6.14	9.25	-2.26	-19.6
EA											
Plasma	2.54	3.30	-	3.30	3.17	2.97	2.94	2.59	3.03	-0.27	- 8.2
Erythrocyte	7.34	12.10	-	12.10	13.06	12.01	10.49	8.44	11.85	-0.25	- 2.0
EA (F)											
Plasma	3.80	3.20	-	3.20	5.30	3.47	3.68	3.19	4.15	+0.95	+29.7
Erythrocyte	8.96	12.50	-	12.50	12.87	11.61	9.79	7.23	11.42	-1.08	- 8.6
EA											
Plasma	3.56	1.60	-	2.58	3.71	-	-	3.10	3.71	+1.13	+43.8
Erythrocyte	13.95	12.22	-	13.08	12.10	-	-	9.79	12.10	-0.98	- 7.5
AA (F)											
Plasma	1.65	3.59	-	2.62	1.58	-	-	1.44	1.58	-1.04	-39.7
Erythrocyte	14.12	13.94	-	14.03	13.46	-	-	11.77	13.46	-0.57	- 4.1

(A) This value was not available from the clinical laboratory.
(B) A second post-exposure sample was inadvertently not taken.

* Data not available at the time this report was prepared by Chemagro Corporation.

Table XII

RESIDUES FROM GUTHION SC IN GLOVES AFTER THE FIRST DAY OF PICKING, 7 HOURS

PARTICIPANT'S INITIAL	MG. IN GLOVES	PERCENT DIFFERENCE	
		PLASMA	ERYTHROCYTE
AT	83.9	-23.5	-35.9
NH	83.5	+33.5	-16.2
JdL	71.7	- 6.4	- 6.1
JM	71.4	+46.1	- 1.8
RP	69.6	-30.5	-23.2
JE	65.2	-25.7	-21.7
EA	54.1	- 8.2	- 2.0
EA	50.8	+29.7	- 8.6
VS	31.3	+19.7	-20.0
MC	29.3	- 5.9	-55.3
ET	26.5	+95.0	-19.6
LR	20.0	-23.5	-18.6

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October 7, 1970

Table XIII

EXPOSURE OF RESIDUES FROM GUTHION SC TO PICKERS

PARTICIPANT'S INITIAL	RESIDUES (IN MG.) FOUND AFTER 1 HOUR EXPOSURE					PERCENT DIFFERENCE	
	5.25 $in.^2$ Each		Gloves	Air		Plasma	Erythrocyte
	Arm	Head		Total	γ/Liter		
JE	0.001	0.000	7.31	0.026	0.140	-25.7	-21.7
VS	0.011	0.007	9.36	0.029	0.160	+19.7	-20.0
RP	0.031	0.004	6.75	0.016	0.089	-30.5	-23.2
AT	0.000	0.009	13.1	0.018	0.097	-23.5	-35.9
ET	0.014	0.008	9.62	c	c	+95.0	-19.6
JdL	0.001	0.007	8.38	0.018	0.097	- 6.4	- 6.1
JM	0.008	0.004	10.0	0.015	0.084	+46.1	- 1.8
MC	0.000	0.007	18.3	0.015	0.084	- 5.9	-55.3
NH	0.032	0.008	3.35[a]	0.008	0.044	+33.5	-16.2
EA	0.009	0.007	b	0.005	0.025	- 8.2	- 2.0
LR	0.007	0.005	b	0.014	0.077	-23.5	-18.6

[a] Gloves were inadvertently "shaken to remove dust" before collection.
[b] Gloves were worn during entire day. Results are given in Table
[c] Analysis is not yet available.

Figure 14

Block 1, GUTHION SC

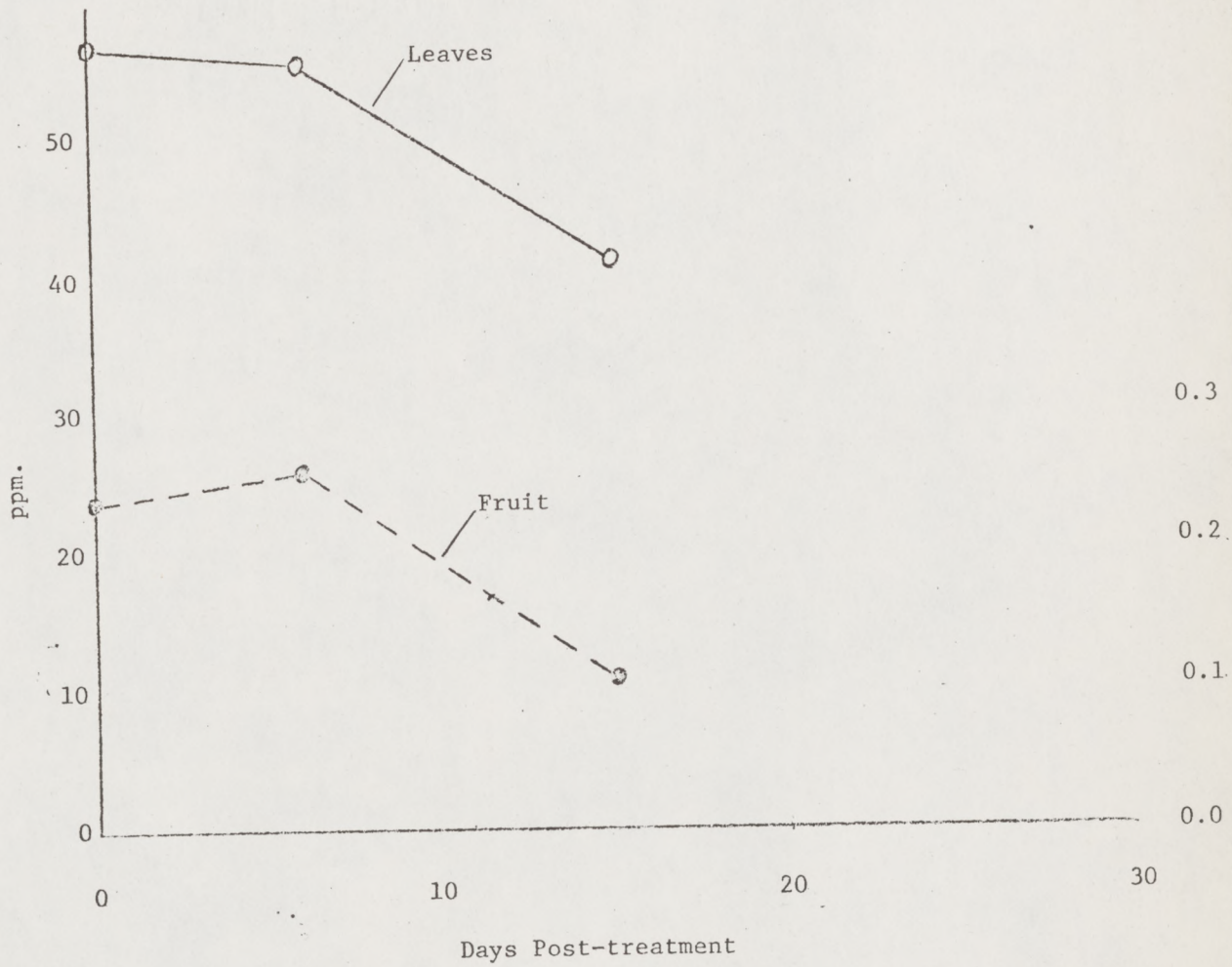


Figure 15

Area A, Block 1, GUTHION SC (Ethion EC)

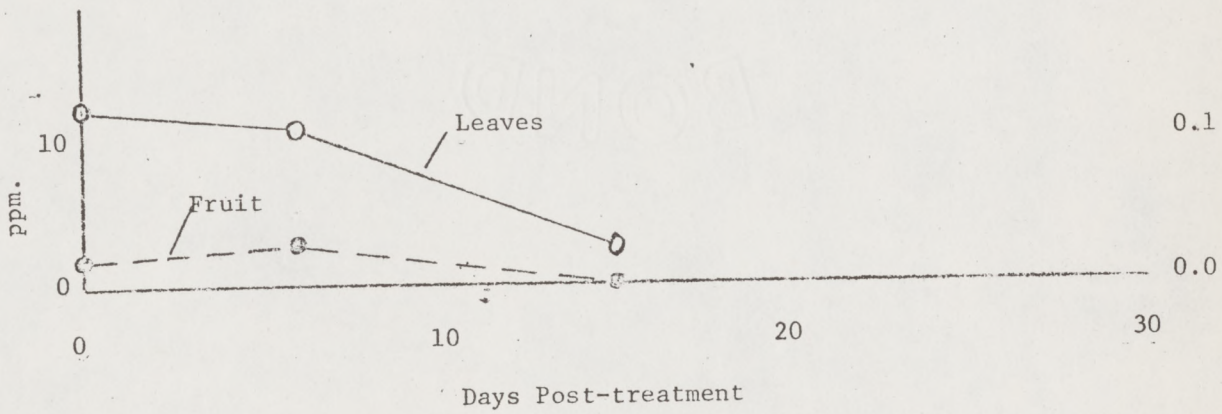


Figure 16

Area A, Block 1, Ethion EC (GUTHION)

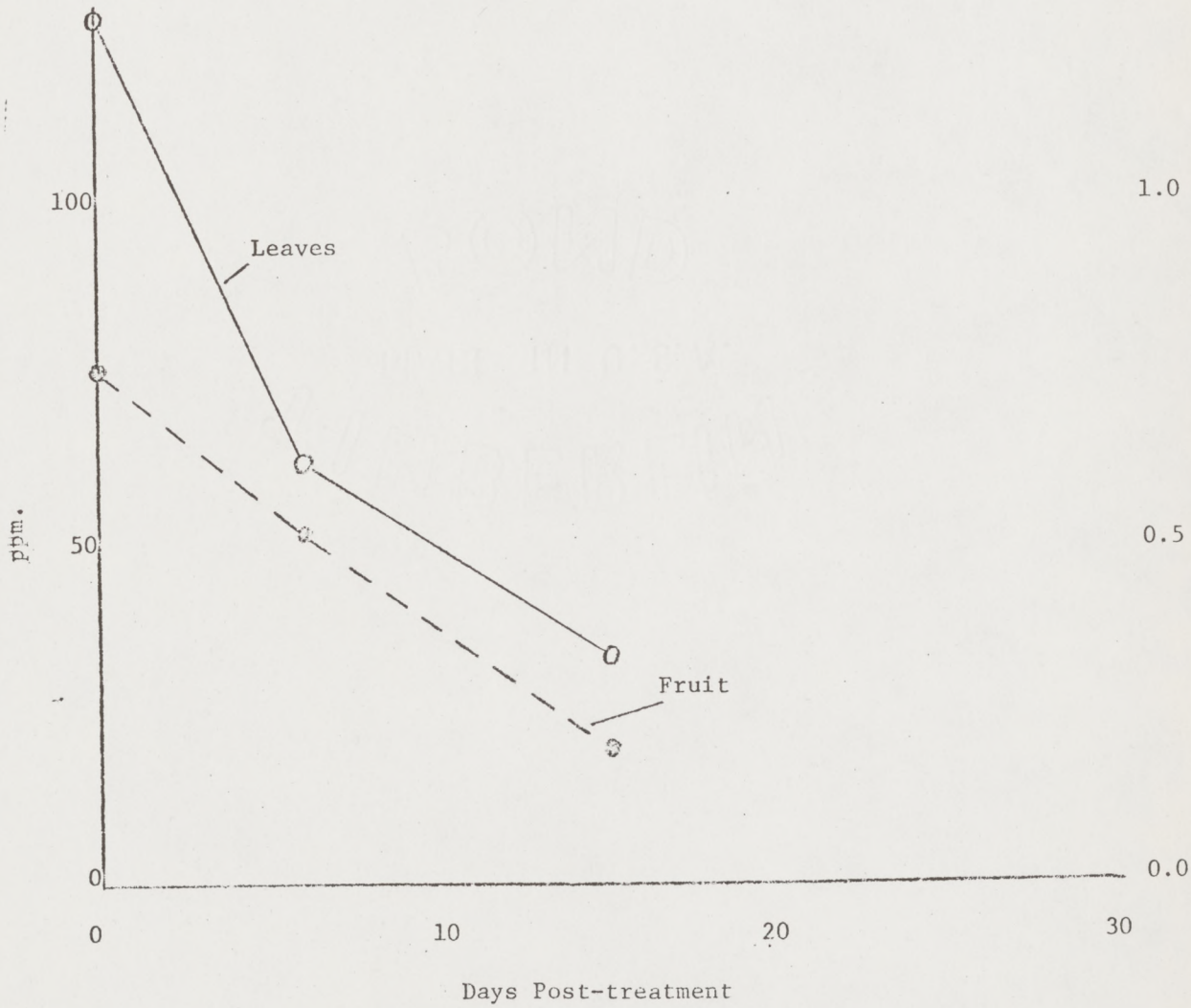


Figure 17

Area B, Block 1, Ethion EC

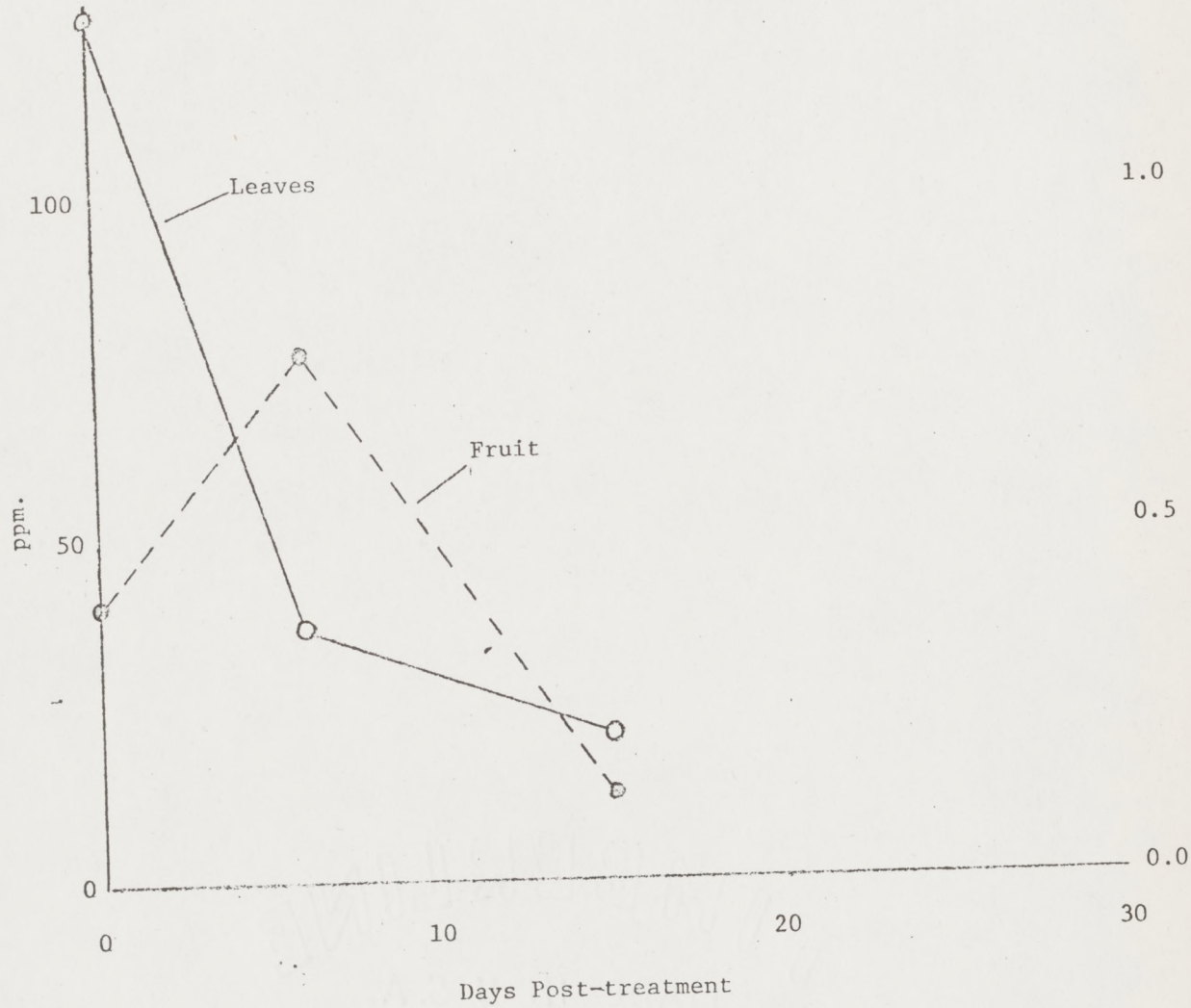


Figure 18

Area C, Block 1, GUTHION SC
1800 gal./acre

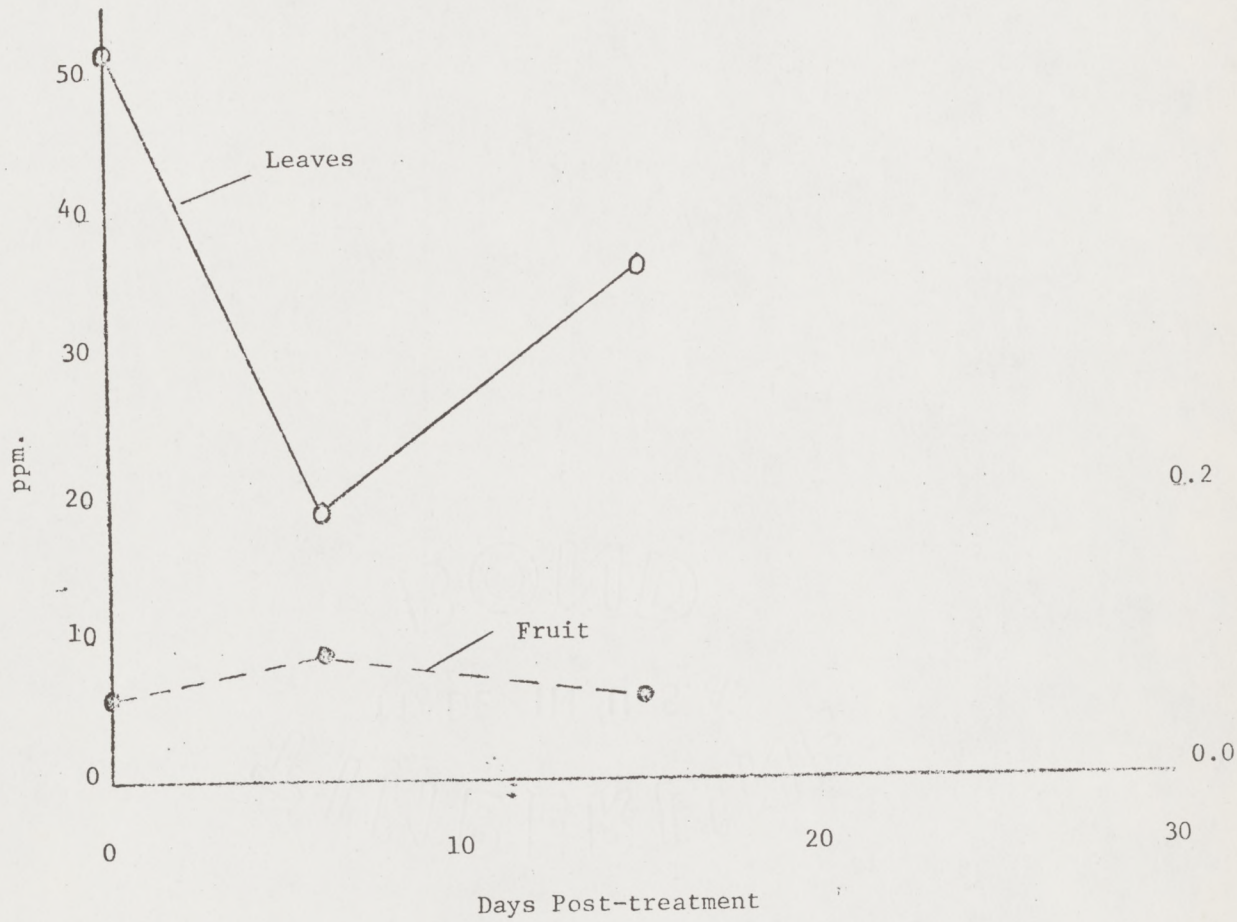


Figure 19

Area D, Block 1, GUTHION SC (Ethion WP)

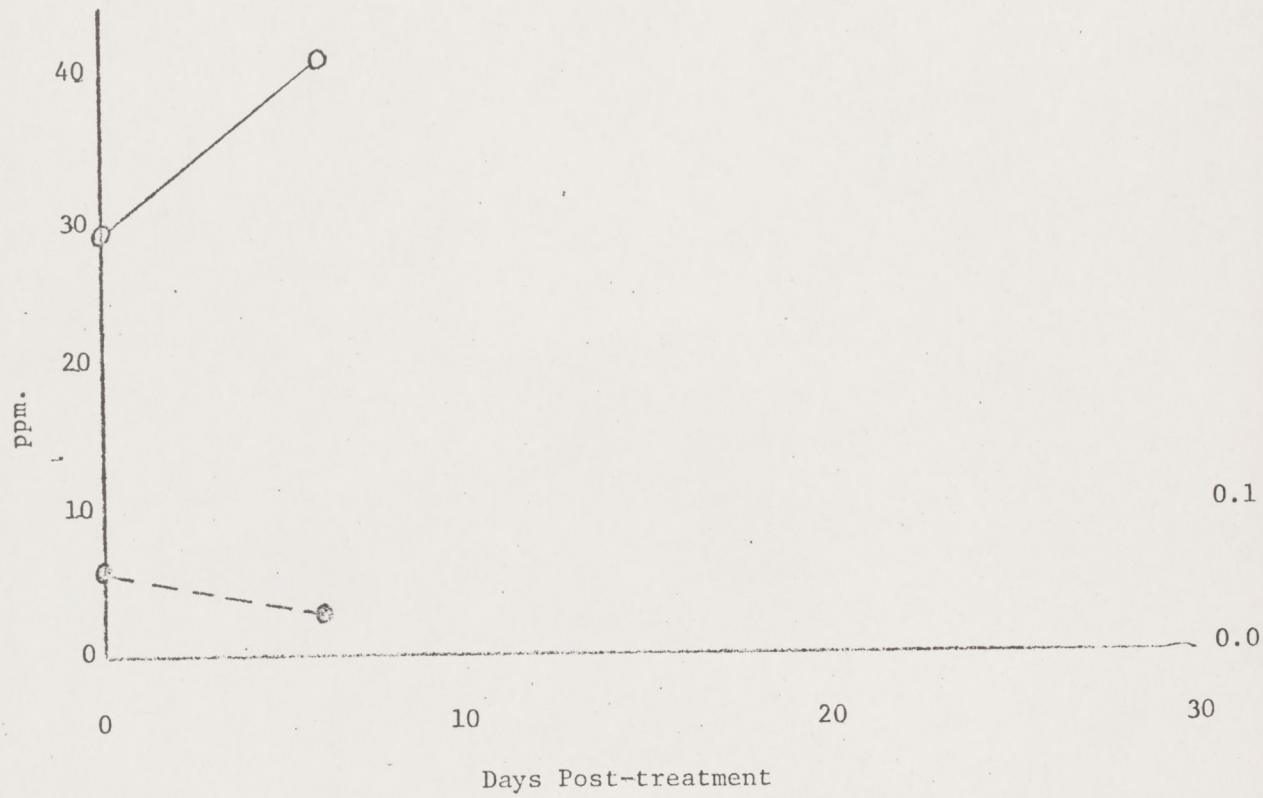


Figure 20

Area D, Block 1, Ethion WP (GUTHION)

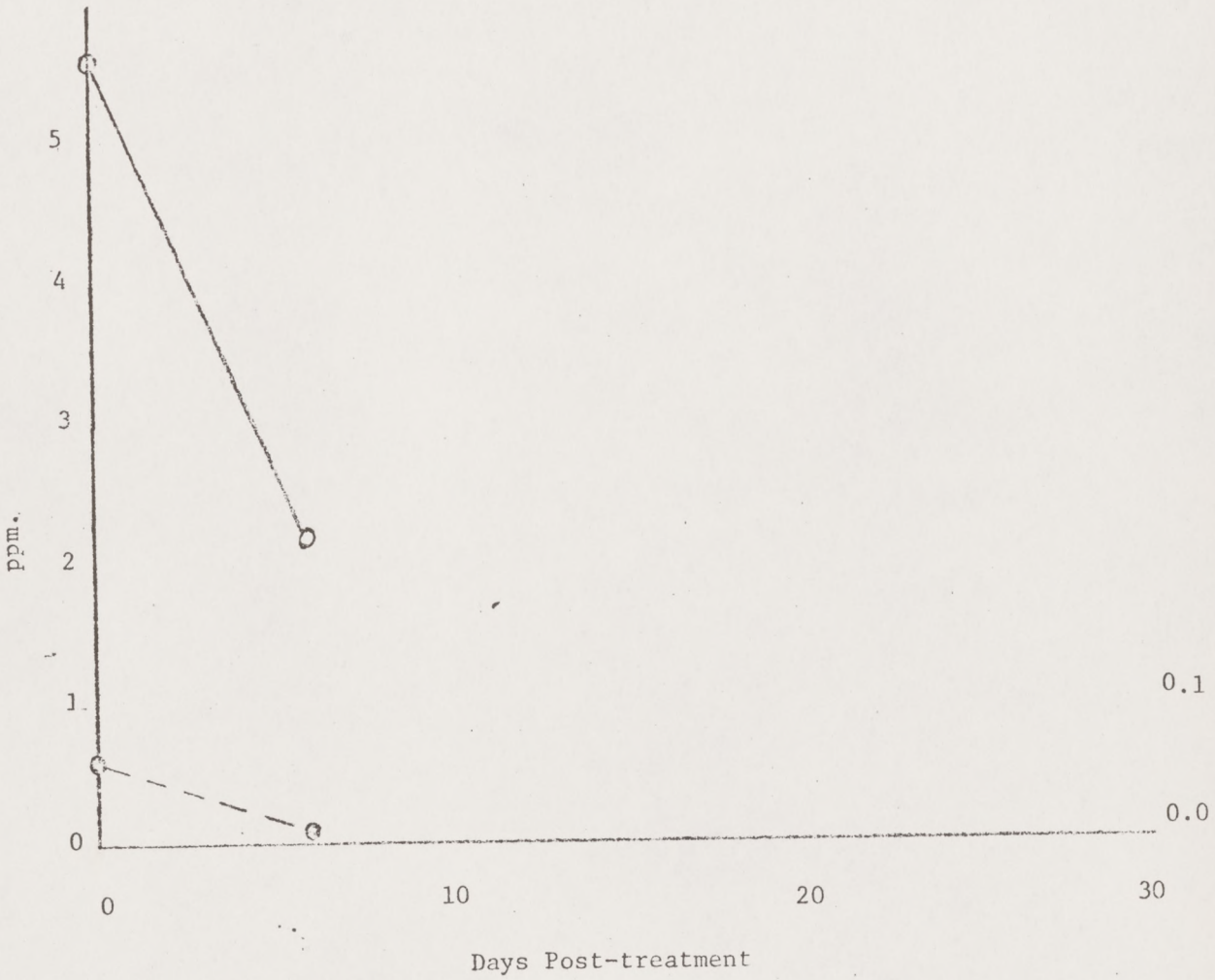


Figure 21

Block 2, GUTHION SC

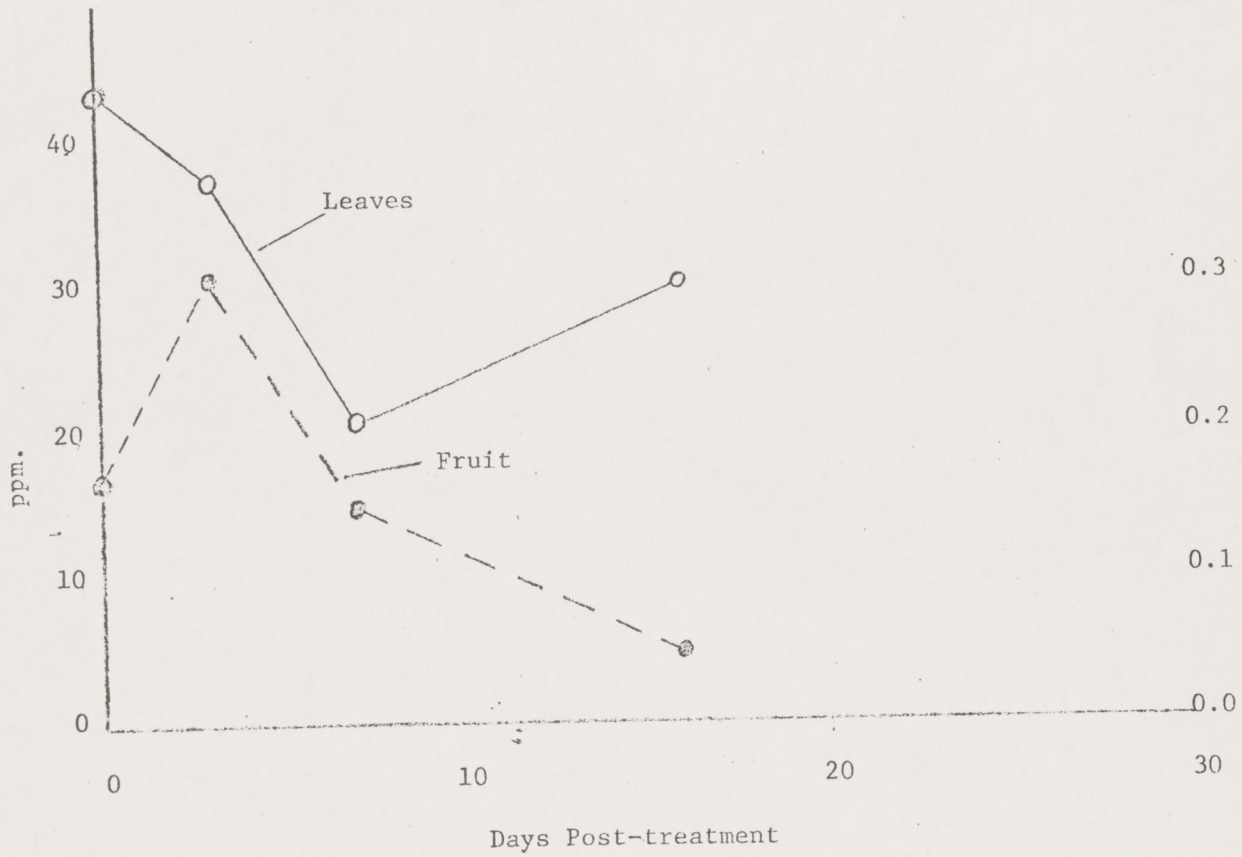
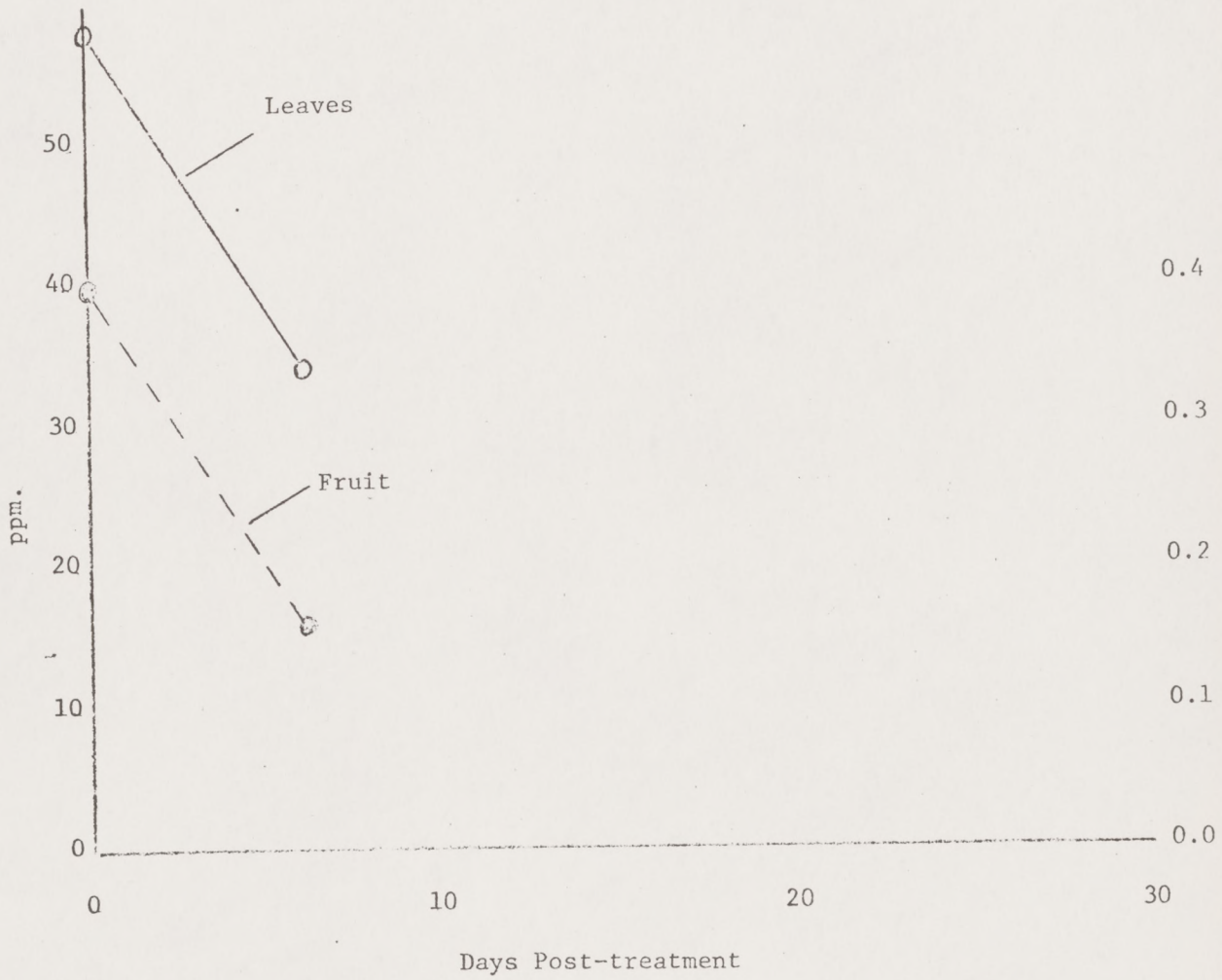


Figure 22

Block 3, GUTHION SC



Anderson

analysis

of

Chicago

FORM 7 (REV. 3-68)

MESSAGE

To_____

Date_____ Time_____ A.M. P.M.

WHILE YOU WERE OUT

Miss
Mrs.
Mr._____

Of_____

Phone_____

Phoned . . . . . ☐	Was in . . . . . ☐
Please call . . . . . ☐	Will call again . . . . . ☐
Will phone again . . . ☐	Would like to see you . ☐
Returned your call . . . ☐	No message . . . . . ☐

Message_____

_____

_____

_____

_____ _____

*Received by*_____

Have you made a suggestion today?
Send your ideas to Merit Award Board

Comments on Chemagro Corporation Research Department, Reports on "Determination of the Hazards to Workers Picking Citrus Treated with GUTHION..."

These studies, of wettable powder and spray concentrate formulations respectively, were relatively well designed by comparison with some others. The number of subjects was rather stable -- about fifteen in each study. An attempt was made to obtain three pre-exposure cholinesterase levels, and at least two levels after exposure. Leaf and fruit surface residue data were collected for thirty days following application of guthion. Residue studies included possible potentiation between guthion and ethion. Climatic information--minimum and maximum temperatures, wind velocity and direction, etc. -- was obtained. In various other ways, the studies were comparatively sophisticated.

Nonetheless, there are grounds for questioning the company's stated conclusion that seven days is an adequate waiting period when guthion is applied in typical forms and quantities (2 1/4 pounds active spray concentrate per acre; 3 3/4 pounds active wettable powder per acre). The company states, with respect to its wettable powder study, "Average post-exposure cholinesterase levels ...were 96.9% (plasma) and 81.3% (erythrocyte) of the pre-exposure average for males and 119% (plasma) and 87.5% (erythrocytes) of the pre-exposure average for females." With regard to spray concentrate, the company states, "Average cholinesterase levels ...were -31.7% plasma and -13.5% erythrocytes for male workers and -52.8% and -16.1% for female workers".

Although plasma cholinesterase levels were in some cases very substantially depressed -- by more than 50% for some groups of workers --verbally, the Chemagro Corporation takes the same position as the Niagara Co. in its earlier study: i.e., that plasma cholinesterase levels have no proven physiological significance. As far as red blood cell cholinesterase is concerned, the Chemagro Corporation takes the position that "Depression of erythrocyte levels was less than the significant level (25%)".

We shall here examine the Chemagro data from the same perspectives described in our discussion of the Niagara data. To recapitulate briefly:

1. Subjects in the "pre-exposure" period were not, in fact, unexposed but were working in various citrus groves where they may have been exposed to a variety of organophosphate residues. Under these circumstances, the closest thing to a "baseline" is the highest value observed in the pre-exposure period, rather than the company's technique of the average value.

2. In a longitudinal study, it is even more inappropriate to average the post-exposure values as the company does. The meaningful measurement is the final measurement.

3. Chemagro Corp., like Niagara, argues that if the total series of subjects shows an RBC cholinesterase depression of less than the "significant level", 25%, the product is safe. This is an improper application

of the 25% concept, which is nothing more than a rough rule of thumb some physicians feel is useful in individual clinical cases. In a statistical series, there are tests far better than any rough rule of thumb to determine whether a change is significant or not. In the present case, involving a cohort of subjects observed over time, the most appropriate statistical test is probably the "Student" or "t-test", with the following formula:

$$t = \frac{\bar{x}_d - \mu_d}{\sqrt{\frac{\sum (x_d - \bar{x}_d)^2}{N}} \Big/ \sqrt{N-1}}$$

This formula will here be applied to the four basic classes of data generated by Chemagro: RBC and plasma cholinesterase levels before and after exposure to guthion wettable powder; RBC and plasma cholinesterase levels before and after exposure to guthion spray concentrate.

Table XIV

PLASMA CHOLINESTERASE LEVELS OF CITRUS PICKERS BEFORE AND AFTER EXPOSURE TO GUTHION WETTABLE POWDER

	PARTICIPANT'S INITIAL	BEST AVAILABLE "BASELINE"	FINAL POST-EXPOSURE MEASUREMENT	CHANGE	PERCENT CHANGE
1	EJ	6.00	3.59	-2.41	-40.2
2	GS	3.66	3.16	- .50	-13.7
3	RG	3.66	3.11	- .55	-15.0
4	PM (F)	3.59	1.29	-2.30	-64.1
5	JV	3.95	1.53	-2.42	-61.3
6	LM	4.89	3.69	-1.20	-24.5
7	MM (F)	2.53	0.86	-1.67	-66.0
8	TR (F)	4.29	1.44	-2.85	-66.4
9	MR (F)	3.26	1.51	-1.75	-53.7
10	JP	4.59	3.50	-1.09	-23.7
11	YG	5.14	3.88	-1.26	-24.5
12	JR	4.10	2.16	-1.94	-47.3
13	GG	2.60	0.79	-1.81	-69.6
14	MO	2.48	0.85	-1.63	-65.7
15	CO	2.41	0.39	-2.02	-83.8
Average		3.79	2.10	-1.69	-48.0

In all cases, these data were based on three pre-exposure tests and a final post-exposure test made twelve days after the application of guthion. As may be seen, every subject's plasma cholinesterase was depressed, some by extraordinarily high percentages and to extraordinarily low values. Applying the "t-test" to the entire array reveals that the changes were significant at something on the order of .00001 level.

Table XV

RED BLOOD CELL CHOLINESTERASE LEVELS OF CITRUS PICKERS BEFORE AND AFTER EXPOSURE TO GUTHION WETTABLE POWDER

	PARTICIPANT'S INITIAL	BEST AVAILABLE "BASELINE"	FINAL POST-EXPOSURE MEASUREMENT	CHANGE	PERCENT CHANGE
1	EJ	15.88	12.99	-2.89	-18.2
2	GS	14.91	12.46	-2.45	-16.4
3	RG	15.62	15.94	+ .32	+ 2.0
4	PM (F)	13.04	8.84	-4.20	-32.2
5	JV	11.57	7.44	-4.13	-35.7
6	LM	14.53	12.46	-2.07	-14.2
7	MM (F)	16.40	12.95	-3.45	-21.0
8	TR (F)	11.12	11.15	+ .03	+ 0.3
9	MR (F)	12.22	7.64	-4.58	-37.5
10	JP	14.70	11.84	-2.86	-19.5
11	YG	12.35	11.46	- .89	- 7.2
12	JR	14.69	15.81	+1.12	+ 7.6
13	GG	12.43	9.45	-2.98	-24.0
14	MO	10.53	6.03	-4.50	-42.7
15	CO	9.23	6.03	-3.20	-34.7
	Average	13.28	10.83	-2.45	-18.4

These data, like those in Table XIV, were based on three pre-exposure tests and a post-exposure value taken twelve days after the orchard was treated with guthion, with one exception: case #7 had a final post-exposure test nine days after application rather than twelve. The over-all changes are significant at the .01 level of confidence.

Table XVI

PLASMA CHOLINESTERASE VALUES GUTHION
SPRAY CONCENTRATE

PARTICIPANT'S INITIAL	BEST AVAILABLE "BASELINE"	FINAL POST-EXPOSURE MEASUREMENT	CHANGE	PERCENT CHANGE
1 MC	2.21	1.53	- .68	-30.8
2 NH	2.99	2.57	- .42	-14.0
3 JdL	3.86	3.06	- .80	-20.7
4 JM	2.73	2.25	- .48	-17.6
5 RP	4.27	2.04	-2.23	-52.2
6 RR	5.01	4.64	- .37	- 7.4
7 LR	3.80	1.23*	-2.57	-67.6
8 VS (F)	3.42	2.13	-2.29	-37.7
9 JE	3.91	2.72*	-1.19	-30.4
10 AT	Insufficient data			
11 ET (F)	Insufficient data			
12 EA	3.30	2.59	- .71	-21.5
13 EA (F)	3.80	3.19	- .61	-16.1
14 IA	3.56	3.10**	- .46	-12.9
15 AA (F)	3.59	1.44**	-2.15	-59.9
Average	3.65	2.50	-1.15	-32.4

* Based on three post-exposure tests.
** Based on two post-exposure tests.

The study design here was a little different from the wettable powder portion of the study. In all but cases 3, 5, and 7, baseline values are based on two pre-exposure tests. On the other hand, there were more post-exposure tests than was true in the wettable powder study. Except as otherwise noted in the footnotes of Table XVI, four post-exposure tests were conducted, the last of which was taken some 21 days after guthion spray concentrate was applied.

According to Chemagro's analysis of the data, plasma cholinesterase levels showed practically no depression for males (-3.1%), and females actually showed an increase after exposure (+19.0%). Our analysis, however, shows that every worker sustained a depression, the average was -32.4%, and taking the series as a whole the changes were significant beyond the .001 level of statistical confidence.

Finally, the changes in erythrocyte cholinesterase levels after exposure to guthion spray concentrate are summarized in Table XVII. These are probably the most important findings in the entire study, since (1) guthion is usually applied to citrus in the form of spray concentrate rather than wettable power; (2) Chemagro, like Niagara before it, chooses to rest its case on red blood cell cholinesterase assays.

Table XVII

ERYTHROCYTE CHOLINESTERASE VALUES
GUTHION SPRAY CONCENTRATE

PARTICIPANT'S INITIAL	BEST AVAILABLE "BASELINE"	FINAL POST-EXPOSURE MEASUREMENT	CHANGE	PERCENT CHANGE
1 MC	11.92	2.68	-9.24	-77.5
2 NH	14.08	4.99	-9.09	-64.6
3 JdL	12.16	6.14	-6.02	-49.0
4 JM	12.61	5.88	-6.73	-53.4
5 RP	12.41	10.88	-1.53	-12.3
6 RR	17.65	10.36	-7.29	-41.3
7 LR	12.03	6.01	-6.02	-50.0
8 VS (F)	13.79	5.88	-7.91	-57.4
9 JE	16.56	7.80	-8.76	-52.9
10 AT	14.20*	5.37	-8.83	-62.2
11 ET (F)	11.51*	6.14	-5.37	-46.7
12 EA	12.10	8.44	-3.66	-30.2
13 EA (F)	12.50	7.23	-5.27	-42.2
14 EA	13.95	9.79	-4.16	-29.8
15 AA (F)	14.12	11.77	-2.35	-16.6
Average	13.57	7.29	-6.28	-46.9

*Based on one test.

Except as noted, all the baselines derive from a minimum of two pre-exposure samples. The post-exposure measurements, as in the case of Table XVI, were taken twenty one days after the application of guthion spray concentrate. Depression of red blood cell cholinesterase was very striking indeed. The changes were all in the same direction and sufficiently consistent in magnitude that the data in Table XVII, taken all in all, are statistically significant beyond the .0001 level of confidence.

Conclusions may be summarized as follows:

1. Appropriate statistical analysis does not corroborate, but, on the contrary, refutes the Chemagro Corporation's opinion that a seven day waiting period is sufficient when guthion is used in citrus in characteristic formulations.

2. The data clearly indicate that even 21 days is insufficient.

3. How much more than 21 days would be required for worker safety is impossible to judge from this study which conducted no cholinesterase tests beyond that date. In all but one subject, both plasma and RBC cholinesterase levels were continuing to decline at the time the fourth and final blood samples were drawn. (I.e., the fourth test was lower than the third.)

4. As was also true in the Niagara study, the following question may be raised: what medical supervision, if any, did subjects receive when their cholinesterase levels were depressed by absolute amounts or proportions which, by general clinical consensus, represent a potential hazard to their health? Eight of the fifteen subjects in Table XIV should have been seen by physician; six of the fifteen in Table XV ; three of the thirteen in Table XVI; and thirteen of the fifteen in Table XVII. How many of them were? What kind of medical surveillance did they receive? How long was it maintained? A thoroughgoing report should contain answers to these questions.

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DEGRADATION OF RESIDUES OF GUTHION AND ETHION
RESULTS OF STATE DEPARTMENT OF AGRICULTURE TESTS

The California Department of Agriculture, Bureau of Chemistry, has also been involved in residue studies of ethion and guthion on citrus, again to supplement data being used in setting realistic reentry times. Following are pertinent comments and results from their report, prepared by Mr. Rex Magee, covering their field tests July 13 to August 13, 1970. Our comments and analyses of their data are included.

A navel orange grove near Corona in Riverside County was selected as the test site. Kelthane and DDT had been applied earlier in the season, but no known pesticide applications had been made for at least 90 days prior to the immediate study.

The grove was divided into 3 areas and 10 trees were selected and marked in each. The first area was sprayed with ethion only, using 5 pounds ethion 25 w(25% active material as a wettable powder) in 500 gallons of water. This amount covered approximately 56 trees or 5/9 acres. Thus about 2.25 pounds active material were applied per acre. (Niagara Chemical Division, in their field test had applied approximately 7 pounds per acre).

The second test area received a mixture of ethion, at the same level as for the first test area, together with guthion, 7.5 pints emulsifiable concentrate (2 pounds active material per gallon) in the 500 gallons water mix. Guthion application, thus, amounted to approximately 3.37 pounds per acre. (Chemagro Corporation applied about 2.25 pounds per acre in their field study). The form of ethion and guthion used in California is largely, if not almost exclusively emulsifiable concentrate.

The third test area received guthion alone at the level mentioned above.

On July 13, each of the 30 test trees was sampled by removal of at least 50 grams of foliage randomly selected. Foliage from each tree was weighed to 50 grams and placed in a quart jar. Hexane, 100 ml., was added to each, jars were shaken for 2 minutes, allowed to stand 2 minutes, and the same cycle repeated for ½ minute intervals. It was deemed that this procedure removed surface-bound pesticide. Each extract was then analyzed by a Varian Model 200 GLC using a 5 ft X 1/8 in. pyrex column packed with 10% DC - 200 coated on 80/100 in. gas chrome Q. A Varian thermionic detector was employed.

The grove was sprayed on July 14 and resampled by the same procedures on July 15, July 19, July 29, August 3 and August 13.

A summary of the results appears in Table XVIII.

We have subjected these figures to a kinetic analysis similar to that employed for Niagara's data on ethion (Fig. 23). To be noted at first glance

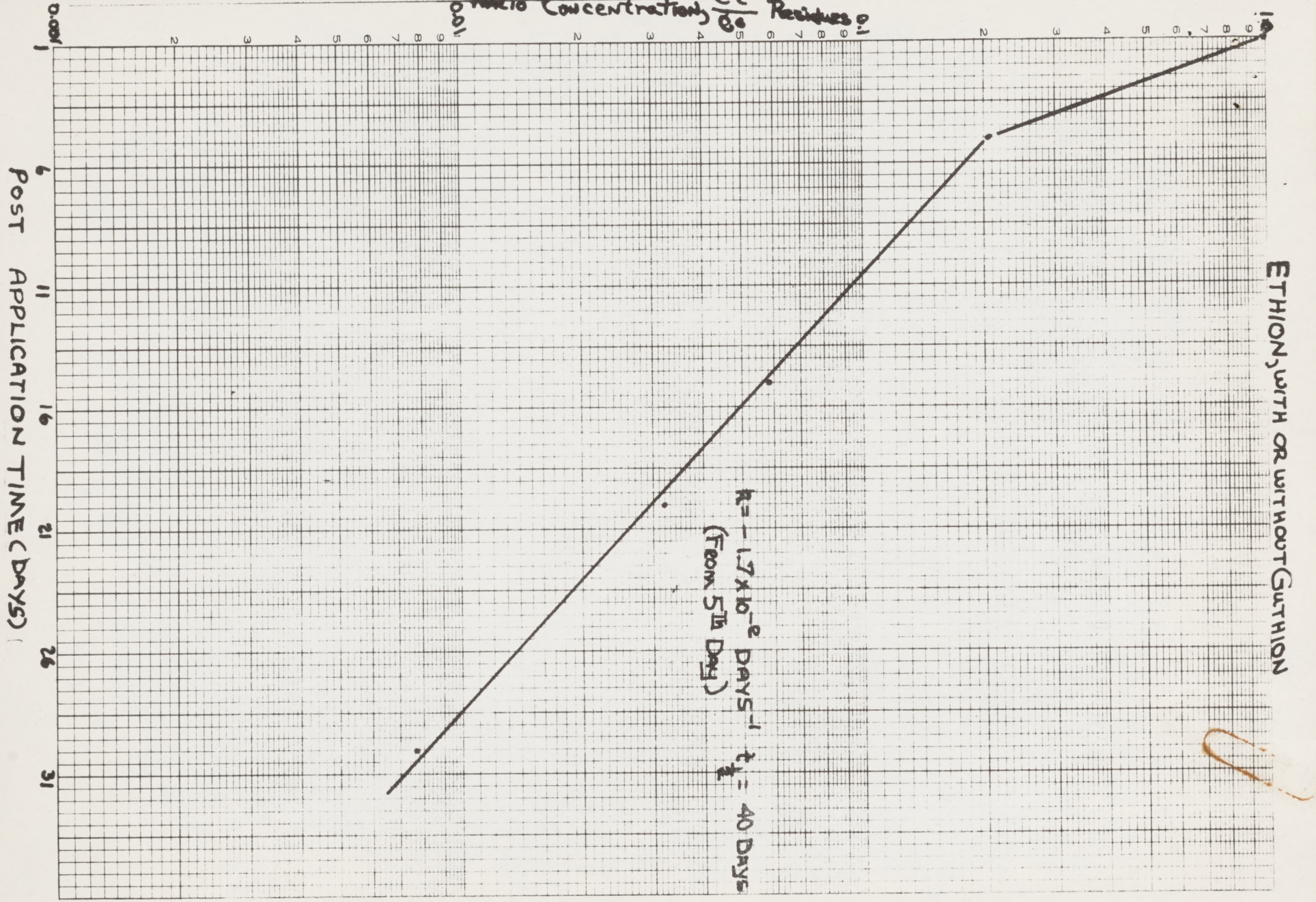

Fig. 23 Rate of decay of ethion, wettable powder, on citrus leaves. Data from California State Department of Agriculture. Co and Ct refer to concentrations at one day post-application and at time t, respectively.

is the extensive loss (80% of pesticide over the first 5 days post-application. Similar results were noted during the Chemagro study. On the other hand, Niagara's data indicated only a 48% loss of the applied dose over the same interval. However, as noted before, the methods of residue extraction differed, as the Department of Agriculture used hexane, Chemagro used water, while Niagara used benzene. The former believes that hexane removes surface-bound residues while benzene extraction of ground leaf tissue would presumably remove residue within, and solubilized on cuticle bearing tissue. Results suggest this may well be the case because; if only surface-bound residue is removed, the effects of photochemical activity, erosion, evaporation, washing, etc. would play a proportionally greater role in minimizing the residual pesticide. Department of Agriculture tests indicated that a 40% higher reading would have resulted for each ethion determination had benzene replaced hexane.

From the fifth to the thirtieth day post-application the loss of the remaining 20% applied material follows first order kinetics with a rate constant of -1.7×10^{-2} days $^{-1}$. Niagara's data indicated a specific rate constant of -4.8×10^{-2} days $^{-1}$. This difference is probably real but not reconcilable with fact. If hexane does remove mainly the surface-bound material one might expect a more rapid first order loss than for the case where leaves were ground with benzene.

As Fig.23 also indicates, ethion wettable powder applied with guthion emulsifiable concentrate does not affect the rate of ethion loss. The presence of ethion, however, does affect the loss of guthion. This observation is in contrast to that of the Chemagro Corporation.

Fig.24 depicts the loss of applied guthion both in the presence or absence of ethion. Following the more rapid initial loss of some 35% over the first 5 days, the decay of residual material probably is of first order, although the arrays of points are not so distinctly linear as are those representing ethion loss. Of interest is the increased loss of guthion in the presence of ethion. Rather than chemical synergism, it may well be that guthion adsorbed onto the ethion powder is more subject to the environmental forces mentioned before, which are expected to account for greater relative losses of surface-bound material.

Other reasons for the increased decay of guthion in the presence of ethion have been suggested by Agriculture: guthion might degrade faster in the presence of ethion; foliage from the third area (guthion only) yielded only 500 in.^{2} per 50 gm sample whereas 600 in.^{2} per sample were measured for the other 2 areas (since results are based on a decrease relative to the initial determination, this possibility seems remote); guthion alone might have been supplied mistakenly at 20% higher concentration than with the mixture. This latter suggestion, as Agriculture points out, would indicate that the rate of loss is a function of initial concentration - precisely inherent in the theory of first order decay. But since the specific rate constants differ for pesticide loss after the two types of application, this possible error during application of guthion alone would not be the only factor involved.

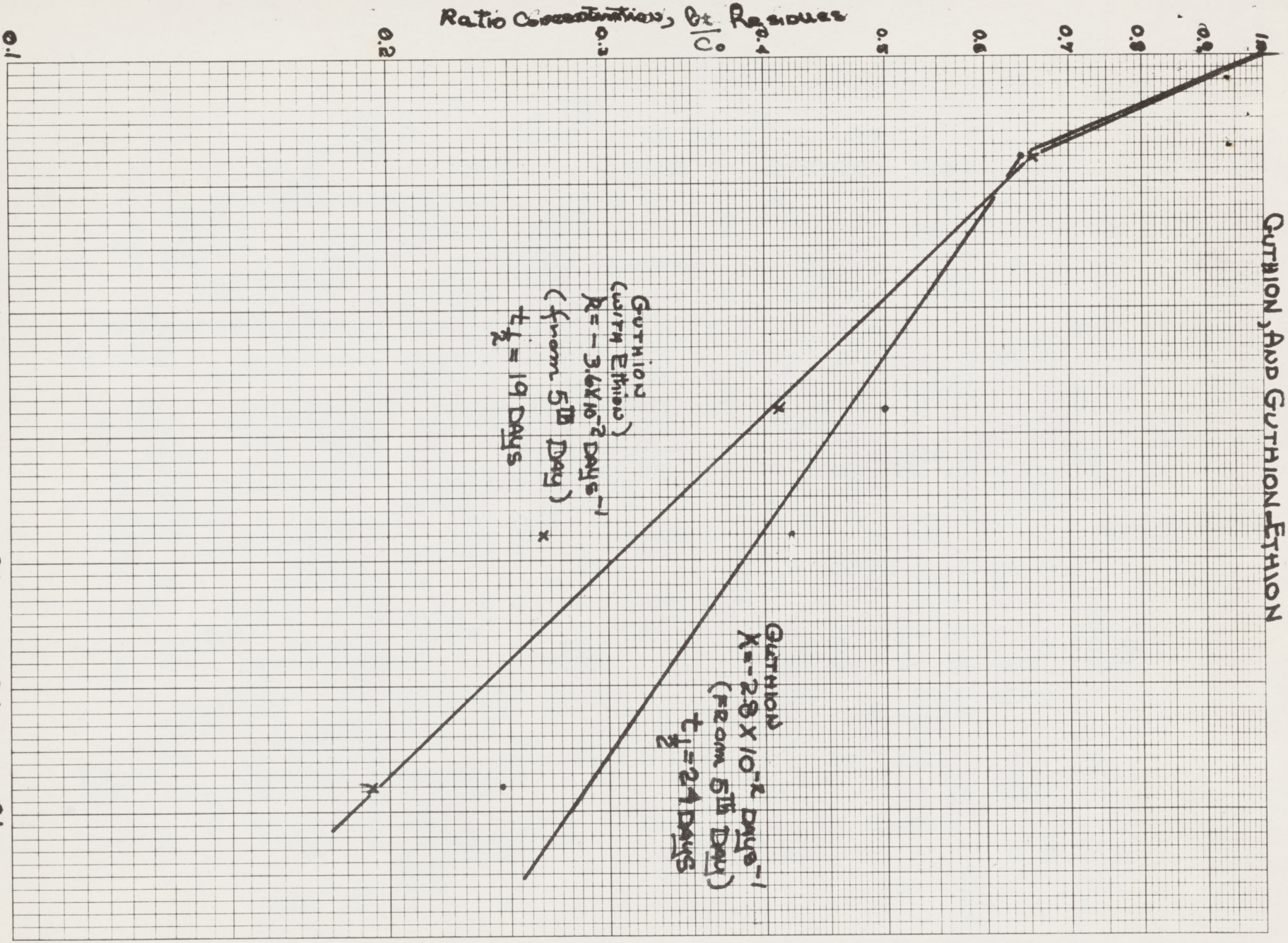

Fig. 24 Rate of decay of guthion, emulsifiable concentrate, alone and in combination with ethion, wettable powder, on citrus leaves. Data from California State Department of Agriculture. Co and C_t refer to concentrations at one day post-application and at time t, respectively.

Tests with guthion indicated a variable increase in residue values ranging from 13% to 34% if benzene replaced hexane during extraction.

As suggested by Agriculture, the methods used permitted an important omission of data - the failure to determine chemicals other than phosphorus - containing compounds. Inasmuch as DDT and Kelthane were applied earlier in the year, some of these residues were probably present. What effect these substances might have on the decay of the organophosphates is unknown.

An estimation of the reliability of the sampling procedures used in the Agriculture study can be ascertained from Fig. 25.

Table XVIII

COMPARISON OF RESIDUES BY PPM AND PERCENTAGE

TEST		DAY 1	DAY 5	DAY 15	DAY 20	DAY 30
Ethion Only	P.P.M.	20.57	4.21	1.20	.66	.16
	%	100%	20%	.05%	.03%	.008%
Ethion	P.P.M.	25.32	5.65	1.43	.77	.17
	%	100%	22%	.05%	.03%	.007%
+						
Guthion	P.P.M.	42.31	27.65	17.38	11.27	8.23
	%	100%	65%	41%	26%	19%
Guthion Only	P.P.M.	53.30	34.16	26.66	22.42	13.13
	%	100%	64%	50%	42%	25%

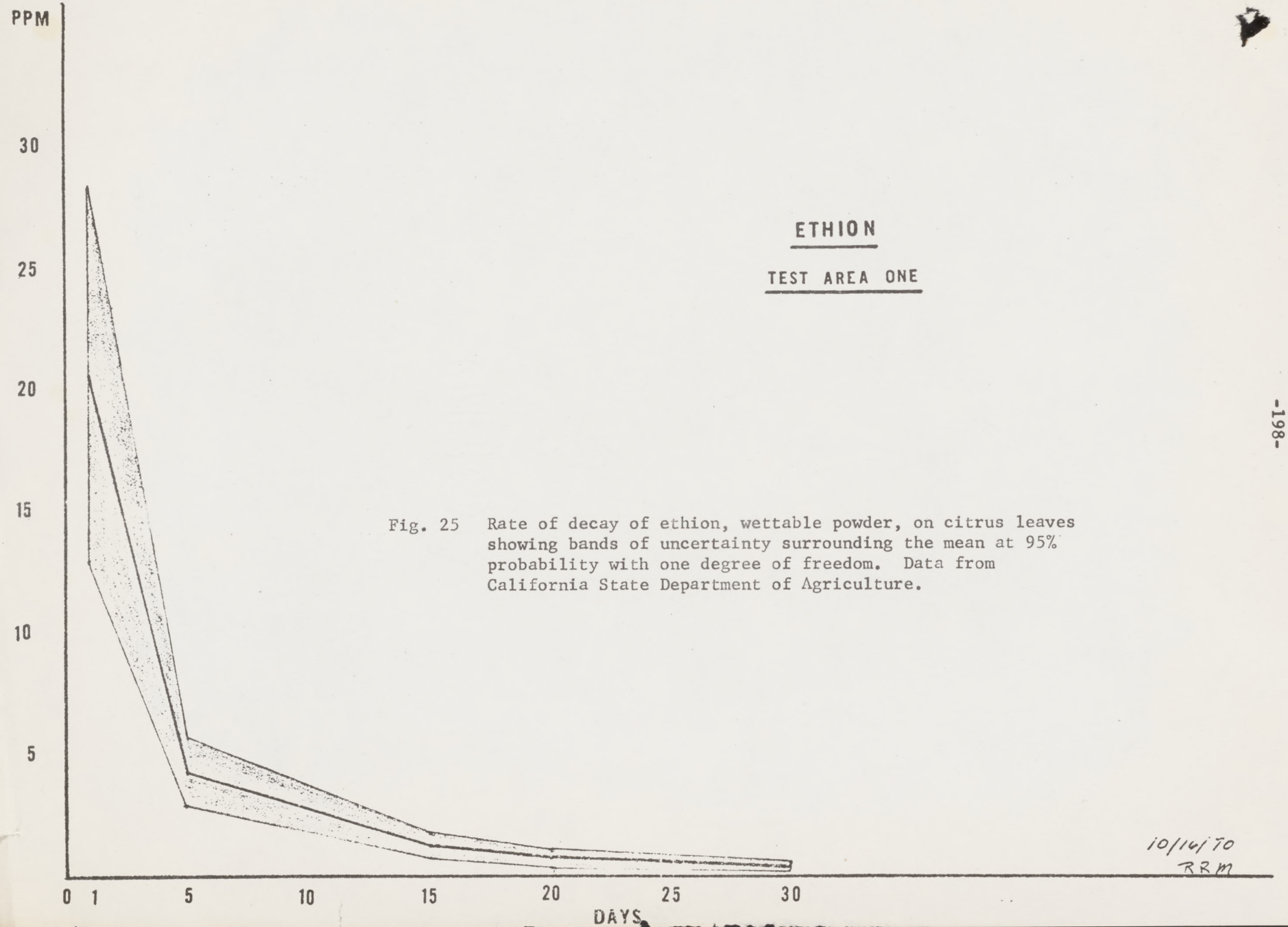

Fig. 25 Rate of decay of ethion, wettable powder, on citrus leaves showing bands of uncertainty surrounding the mean at 95% probability with one degree of freedom. Data from California State Department of Agriculture.